SAMPLING PROTOCOLS FOR THE RAPID BIOASSESSMENT OF STREAMS AND LAKES USING BENTHIC MACROINVERTEBRATES

Second Edition

APRIL 1998

Ontario

Ministry of the Environment

SAMPLING PROTOCOLS

FOR THE RAPID BIOASSESSMENT OF

STREAMS AND LAKES

USING BENTHIC MACROINVERTEBRATES

Second Edition

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ISBN 0-7778-7378-8

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PIBS 3519E01

EXECUTIVE SUMMARY

Rapid bioassessment protocols have been developed as inexpensive screening tools to assess the biological health of the macroinvertebrate communities of streams and lakes. This report summarizes rapid bioassessment methods currently used by the Aquatic Science Section of the Ontario Ministry of the Environment. These protocols are being evaluated to ensure their general utility, accuracy and repeatability. In the future these simple methods may be used by the general public to establish whether a given stream or lake is healthy or degraded.

Historically, benthic macroinvertebrate collections were used as indicators of the biological impacts of degraded water quality. Samples of mud, debris and associated invertebrates were generally sorted in their entirety and the animals were identified to the species or genus taxonomic level. However, these types of exhaustive censuses are time consuming and expensive. In recent years rapid bioassessment techniques have been introduced as cost-effective screening tools. Rapid bioassessments are often based on a subsample of 100 animals that are identified to a very coarse taxonomic level such as order rather than species or genus. This reduction in taxonomic detail and increased subsampling greatly reduces the cost, expertise and amount of time required for biological assessments. As a result, rapid bioassessments provide an opportunity for inexperienced volunteers to undertake biological assessments.

This series of protocols describes methods for conducting rapid bioassessments in the shallow waters (i.e., <0.5 m depths) of streams and lakes. This report provides guidance regarding the time of the year to sample, how to select different types of biological indices, and how to evaluate a test site with respect to regional reference values. Example calculations and blank bench sheets are included. Ongoing studies will establish if rapid bioassessment protocols can be used by inexperienced volunteers to identify potentially impacted areas where detailed follow-up studies may be warranted. Thus, rapid bioassessment methods are intended to be used as simple, cost-effective screening tools that can distinguish healthy sites from those sites where further investigation may be necessary.

TEN DOs AND DON'Ts FOR RAPID BIOASSESSMENTS

1. Do ask permission to access a stream or lake site; don't trespass

2. Do choose the 3 stream sites (or 5 lake sites) carefully; sites should represent the most common habitats (bottom types); don't pick the extremes

3. Do ensure that the bottom types are recorded on the benthic enumeration sheets; animals found at a site comprised of sand and no vegetation will be very different from the fauna inhabiting sites with plants and woody debris or cobbles

4. Don't lily-dip when collecting a sample; be aggressive and scour the bottom to ensure that sedentary animals and associated debris are collected; don't just filter the water; most animals are associated with plants, debris and sediments

5. Do collect lots of bottom material and plant debris to ensure that more than 100 animals are captured; don't give up easily because samples with less than 100 animals are of limited value

6. Do turn over rocks and logs within the sampling area in order to dislodge and collect animals that are hidden underneath or attached to the bottom

7. Do ensure that at least 4-or-5 random subsamples of the collected debris are sorted in order to obtain 100 animals; don't forget to subsample the material at the bottom of the sample because many animals will burrow in an attempt to hide; remember that random *means* random

8. Do ensure that the enumeration sheets and associated vials are clearly identified with the essential site information and date of collection; don't use ink since it runs; **samples are of no value if their origin is unknown**

9. Do collect samples from reference (healthy) streams or lakes in the vicinity of the sites in question in order to characterize the benthic community in areas with acceptable water quality (i.e., to provide regional reference sites)

10. Do use a variety of biological indices or metrics to assess the health of a given body of water; different indices respond to different stressors in different ways; a single index is of limited value

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INTRODUCTION

Macroinvertebrates (i.e., large bottom-dwelling insects and related invertebrates found in streams and lakes) are relatively sedentary, exhibit varying tolerances to physical and chemical changes and are widespread in their distribution. As a result, macroinvertebrate collections have been used extensively as a means to assess degraded water quality in streams and lakes (Wilhm and Dorris 1968, Barton and Metcalfe-Smith 1992, Metcalfe-Smith 1994). Although field samples are relatively easy to collect, it is generally time consuming and labour intensive to identify all of the animals to the species or genus taxonomic level. Consequently, the laboratory processing of a large number of samples can be expensive.

The recent development of rapid bioassessment techniques (e.g., as utilized by the United States Environmental Protection Agency; Plafkin et al. 1989) capitalizes on the experience gained from complete macroinvertebrate enumerations, but permits the sampling of a large number of streams or lakes at a much lower cost (e.g., Hilsenhoff 1988, Resh and Jackson 1993, Chessman 1995, Resh et al. 1995). This saving is achieved by extensive subsampling and only identifying the animals to the coarse taxonomic level of order (or higher).

One goal of rapid bioassessment protocols is to provide an inexpensive, relatively simple screening tool that can be used by people with little or no expertise in aquatic ecology (e.g., Firehock and West 1995, Lathrop and Markowitz 1995, Penrose and Call 1995, Culp et al. 1997). The protocols that follow are experimental rapid bioassessment methods that were developed to complement standard field procedures employed by staff at the Dorset Environmental Science Centre of the Ontario Ministry of the Environment (e.g., Girard and Reid 1990, Clark 1996, Reid et al. 1997). These protocols are a blend of methods used by other jurisdictions (e.g., Plafkin et al. 1989, Shaw et al. 1992, Resh et al. 1995, Stanfield et al. 1996).

The following protocols focus on sampling the shallow areas of streams and lakes (i.e., waters less than 0.5 m in depth). Although it may be impractical for inexperienced people to sample the macroinvertebrates found in deeper waters, the principles underlying rapid bioassessments may also be applied to deep-water collections based on more traditional equipment such as grab samplers or artificial substrates (e.g., see Bode et al. 1990, Klemm et al. 1990).

Results of rapid bioassessments can be used to assess the biological health of a given stream or lake by direct comparison with data from regional reference sites. Test sites that fall outside of the normal range of biological variation will be candidates for follow-up assessments involving more-detailed taxonomic information (e.g., Bode et at. 1990, 1995, Griffiths 1993). Water-quality testing may be warranted if the detailed biological assessment confirms the existence of degraded or impaired conditions.

The rapid bioassessment protocols described in this document are currently being ground-truthed to determine whether the resultant data have sufficient accuracy and precision to detect known differences in the water quality of streams and lakes. Further changes may be implemented following this trial period.

METHODS

Stream and Site Selection - Streams are chosen according to the specific requirements of the assessment. Local Conservation Authorities and the Ministry of Natural Resources should be contacted before sampling a given stream. Each agency is usually interested in biological assessments within their jurisdictions and historical data for the sites of interest may be available. In addition, staff from these agencies can help determine if the proposed sampling sites are on private property. If so, arrangements should be made with the landowner to ensure safe access to the sites.

Within a given stream, we recommend sampling riffles (i.e., fast-flowing areas) because of the high productivity and high diversity of invertebrates associated with this type of habitat (as compared to runs and pools; Bode et al. 1990). If no riffles are found, then debris dams and snags provide a reasonable substitute. Water depths should be less than 1 metre. Three riffles are selected as representative sites, with variable distances between them depending on stream morphology. Once these 3 sites are chosen, sampling begins at the site furthest downstream so that the upstream sites are undisturbed until sampled.

If the water conditions are unsuitable for kick-and-sweep methods (e.g., large, fast-flowing rivers where wading may be unsafe), we recommend the use of traditional sampling methods such as grab samplers, corers or artificial substrates (e.g., see Klemm et al. 1990). Many types of grab samplers or corers are too expensive and too heavy for inexperienced volunteers to purchase and use safely. As an alternative, Bode et al. (1990) provide details for the construction and proper use of artificial substrates. Briefly, artificial substrates are simple devices constructed from hardboard that are suspended in the water column for a period of 5 weeks. During this period macroinvertebrates colonize the artificial substrates. These animals are subsequently enumerated when the artificial substrates are collected. The resultant bench sheets can be compared to data from artificial substrates deployed at regional reference sites. Because this approach provides a uniform substrate or habitat for the colonizing animals, this technique may also be warranted if the habitats at a test site are unique when compared to regional reference sites.

Lake and Site Selection - Lakes are also chosen according to the requirements of the assessment. Much like the stream protocol, local Conservation Authorities and the Ministry of Natural Resources should be contacted before undertaking a rapid bioassessment. In some instances, historical data may be available. Staff from these agencies may also assist in determining the best access points and

whether potential sampling sites are on private property. If so, arrangements should be made with the landowner to ensure safe access to the sites.

Because lakes tend to include a wider variety of habitats than streams, we recommend that 5 representative sites should be sampled in a given lake. These 5 sites should be randomly selected from a series of candidate sites in proportion to the different types of nearshore habitats. For example, if aquatic plant or macrophyte beds were found in 60% of the nearshore areas, then 3 of the 5 sites should have macrophytes. In some cases, especially with small lakes, it may be difficult to find different habitats. In this instance all 5 sites may comprise a single substrate such as silt or sand. Other types of common substrates include boulders, cobbles, gravel and organic debris or detritus. Remember that it is important to choose sites that are as representative or characteristic of the lake as possible.

Large lakes can present a different set of problems. Aside from offering a wide variety of habitats, the predominant bottom types may be relatively unproductive sand, gravel and cobble beaches. Sampling in these areas may result in very few animals. In our limited experience, the greatest numbers of macroinvertebrates are captured in areas with soft bottoms and plants or woody debris that provide shelter from wave action and predatory fish. Consequently, the number of animals that is collected often reflects the amount of debris that is removed. Several litres of mud, plants and debris should be collected from a given site in a lake to ensure that sufficient numbers of animals are obtained. Typically, very little material can be collected from a rocky shelf or cobble area. As a result, you must balance two objectives when sampling the shallows of large lakes. The first objective is to sample representative habitats; the second is to ensure that at least 100 animals are collected. Priority should be given to collecting as many animals as possible. Thus we recommend sampling softer substrates and areas with plants and woody debris in larger lakes.

In lakes where nearshore substrates are not suitable for kick-and-sweep sampling, it may be necessary to utilize a different approach such as artificial substrates (see Bode et al. [1990] for details). Alternatively, soft sediments in the deeper parts of the lake may be sampled with various bottom-grabbing devices or corers (e.g., see Klemm et al. 1990). Because these types of samplers select for a different benthic invertebrate community, regional reference data must be collected with the same equipment (e.g., see Appendix 5 for a comparison of grab samples with shallow-water kick-and-sweep samples).

Sampling Procedures - Numerous rapid bioassessment techniques are currently in use (e.g., Bode et al. 1990, Resh and Jackson 1993, Resh et al. 1995, Culp et al. 1997). Among the various methods, there are differences in the recommended number of invertebrates that should be removed from each sample (as well as the number of sites) in order to provide a reasonable estimate of the water quality

Equipment List

- road and/or topographic maps
- 3 benthic enumeration sheets per stream (1 for each riffle) **OR** 5 benthic enumeration sheets per lake (i.e., 5 sites; see Appendix 2)
- thermometer (alcohol type, not mercury in case of breakage)
- meter stick
- pencils and/or china markers
- tape (for labelling the outside of the vials)
- waterproof paper (labels to be placed inside the vials if possible)
- a seine net with two poles (60 x 110 cm 1.6 mm mesh (window screen) attached to two 2 cm wooden dowlings with staples, making a 60 cm high x 100 cm wide net). Top and bottom edges of the screen should be reinforced with duct tape before attaching it to the dowling (for further construction details and a picture see http://www.people.virginia.edu/~sos-iwla/Stream-Study/StreamStudyHomePage/StreamStudy.HTML)
- at least 3 plastic buckets (1 for the sample, 1 for rinse water and 1 for the washed subsample)
- squeeze bottle for water
- white basin for sorting and removing the animals from the subsample (may simply use a shallow cake pan painted white)
- fine-tipped forceps
- small pieces of screen (e.g., 2 cm x 8 cm) for "scooping" invertebrates that swim along the surface of the water in the subsample
- guide to distinguishing characteristics of aquatic invertebrates (Appendix 1)
- container of 95% ethanol (may be difficult to obtain); **OR** 70% isopropanol or "rubbing" alcohol
- glass or plastic vials for preserving organisms (or similar small bottle that can be sealed without leaking)

at a given site. Based on current bioassessment strategies (e.g., Plafkin et al. 1989), we use a subsampling technique that requires only 100 animals. Recently we completed a technical assessment of the implications of counting 100, 200 or 300 animals from a series of samples (e.g., see Hannaford and Resh 1995). In our evaluation, we found little gain associated with doubling or tripling the effort to enumerate additional animals (see Appendix 6 for details). On average, it takes about 45 minutes for 2 people to pick 100 animals from a sample. The actual amount of time depends on several factors

including: the size and density of the animals; the amount of fine silt and mud that clouds the sample (note that the fine silt and mud can be removed by rinsing the sample while using the net like a sieve); the amount of organic debris in the sample (e.g., large numbers of leaves can be a problem with autumn collections); and the expertise of the people who sort through the sample and identify the animals. The general sampling procedures for streams and lakes are outlined separately in the sections that follow.

Streams - Benthic invertebrates are collected from relatively shallow riffles with a kick-and-sweep method using a seine net that is stretched between two poles. Two people are required. Sampling is conducted over a one-minute period in an area of approximately 1 m^2. This area may vary somewhat depending on the shape of the stream where the riffle is located. In some cases the stream may be too narrow, so the length of the area sampled would be longer than 1 metre to compensate for the decrease in width. The water depth should be less than one-half metre.

The two poles of the seine are held by the person standing downstream of the net. The bottom ends of the two poles are held against the bottom of the stream and the top of the net is angled downstream in order to form a pocket where the animals and dislodged debris will be collected. Do not stretch the net tightly between the poles, but let the net billow in the current. Once the target area upstream of the net has been identified, the second person stirs up the substrate by vigorously kicking the stream bottom. The net should be lifted periodically to prevent clogging and to shake the invertebrates and associated debris into a bucket. Do not attempt to sample the entire 1-m^2 area with the net in one position. Best results are obtained if the area immediately in front of the net is disturbed, the collected material is dumped in the bucket and then the two-person team moves upstream to the next part of the 1-m^2 area. Several small collections are better than a single large one. Extra time may be required to scrape the bottom of rocks and logs if these types of obstacles are found within the riffle.

Lakes - Benthic invertebrates are collected from each of 5 representative sites in a given lake using a kick-and-sweep method and a fine-mesh seine net. Sampling is conducted over a ten-minute period, being standardized to a constant period of time rather than a standard unit of area (e.g., Okland and Okland 1979, Reid et al. 1995). During this ten-minute time period a variable number of transects are sampled. Each transect is roughly perpendicular to shore running offshore to a depth of approximately three-quarters of a metre. The actual length of the transects will vary depending on the slope of the lake bottom.

Two people are required. One person kicks and dislodges debris and associated animals from the bottom while the second person uses the seine net to collect this dislodged material. This second person should hold one pole of the seine net in each hand. Although the net is roughly one-metre wide, we

suggest rolling it up to only one-half metre in width so it is easier to manage. The net should billow loosely between the two poles to form a pocket. A stout cord can be tied between the two poles at the top and bottom of the net to ensure that the unravelled portion of the net hangs from the poles to form a pocket.

The person who is going to kick the bottom (i.e., the "kicker") walks away from shore to a depth of one-half to three-quarters of a metre. After turning to face the shore, this person begins to kick the sediments and gradually walk towards the shore. As the first person kicks the sediments, the second person should stand at right-angles to the transect and use the two-poled seine net to scoop up the dislodged material immediately in front of the kicker. The person with the net must push the bottom of the seine along the lake bottom because the animals and debris settle out of the water column very quickly. During this sweeping motion, the seine net should be almost parallel with the sediments and often entirely underwater. The dislodged macroinvertebratesand associated debris caught in the net should be drained of water and shaken into a bucket that is carried by the kicker. This procedure is repeated along the length of the transect until reaching the shore. Additional transects must be sampled until the ten-minute time period is completed. The number of transects sampled and the approximate length of each transect should be recorded on the benthic enumeration sheets. Much like the stream protocol, when rocks or logs are encountered they should be scraped off to collect any attached invertebrates.

Recording Site Location and Site Description Information - After the sample is collected, a physical description of the site should be recorded on the benthic enumeration sheet (e.g., stream width, depth, length and riffle location). Site locations should be marked directly on maps or a small sketch should be made on the back of the bench sheet (e.g., see Appendix 3). For lakes, the number of transects and the length of the transects sampled at each site should also be recorded, as well as the specific location of each site with respect to established landmarks (i.e., roads, bridges, buildings, etc.; see Appendix 4). At this time, air and water temperatures should also be recorded.

Once the water column has cleared, a brief description of the sediments at the site should be recorded. This step is extremely important because the type of sediment (or substrate) can influence the type of macroinvertebratesthat are found. Substrate types are listed at the top of the benthic enumeration sheets (see Appendix 2). Where several types of substrates are found at a site, approximate percentages of each substrate should be recorded (see Apps. 3 & 4). The presence and percent cover of aquatic plants (submersed or emergent) should also be noted. In addition to macroinvertebrates, other animals including fish, amphibians and terrestrial insects should be identified and recorded on the bench sheets. Lastly, the presence of an oil sheen, rust-coloured sediments, or any other unusual aspects of the site should be recorded on the enumeration sheet in order to assist with the interpretation of the results.

Picking the Samples - While sampling a site, the material collected in the seine net should be shaken into a plastic bucket quite frequently. Once the sampling is completed, any material remaining on the net should be rinsed into the bucket with the aid of the squeeze bottle. Because some macroinvertebrates will stick to the net, it is important that any remaining organisms and debris are carefully brushed off the screen into the bucket. Large conspicuous animals may be picked from the net and placed directly into the vial once they have been identified and enumerated. However, the material in the bucket must be randomly subsampled in order to prevent biased results. If the sample is not going to be enumerated immediately, some water should be added to the sample and the bucket should be sealed with a lid and labelled. Keep each bucket in a cool place out of direct sunlight until the animals can be enumerated.

Before subsampling the debris, most of the water should be drained from the bucket. Mud and silt can be rinsed from the sample by using the net as a sieve. Be careful not to lose any of the animals. Small amounts of the remaining debris should be removed from the bucket with a table spoon and placed in a shallow white tray (or cake pan) for sorting (the animals are generally easier to see against a white background). Because many of the animals can be identified according to how they swim or move through the water (see Appendix 1), about 1-2 cm of water should be added to the sorting tray. Remember that small subsamples of debris can be sorted more easily and more efficiently than large amounts. Only one-or-two spoonfuls of material should be sorted at a time.

Let the water in the tray clear and then gradually sort through the debris and remove all of the animals in the subsample. Watch for movement, as some animals will come to the surface and others will try to hide under the debris. Any animals that swim should be scooped from the water using a small piece of screen. Those animals that are hidden among the sediments and debris are removed using tweezers. Be sure to search for cryptic animals such as snails and small clams that sink to the bottom of the tray. Careful sorting is required because animals that are attached to the sediments and associated debris are difficult to see. For example, caddisfly larvae with their associated "houses" constructed from bits of wood, sticks, sand or gravel are often missed.

Each animal that is captured is identified according to the taxonomic categories on the bench sheets (see Appendix 2), then placed in the sample vial and recorded with a tally mark in the appropriate box on the bench sheet. Note that not all of the different groups of aquatic invertebrates are represented on the bench sheet. If some animals cannot be identified or located on the enumeration sheet, simply include them in the vial and make a note of this on the bench sheet under a miscellaneous category. The vial should be labelled and it should contain 95% ethanol alcohol (or 70% isopropanol [rubbing alcohol]) to adequately preserve the specimens.

Once a subsample has been sorted and

all of the animals have been removed, the subsample is discarded and another tablespoon of material is randomly selected from the bucket. Be sure to remove material from both the top and bottom of the bucket since some animals will burrow in an attempt to hide. A minimum of four-or-five randomly selected subsamples are sorted to ensure that a representative set of animals has been removed from the sample. Once approximately 100 animals have been enumerated, the remainder of the sample is discarded.

Ten-or-more subsamples of material will often have to be examined in order to find 100 animals. Occasionally, less than 100 animals are found after the entire sample has been sorted (e.g., in some small streams and unproductive areas in large lakes). This problem usually occurs if the habitat at a site is unsuitable for most invertebrates (e.g., substrates such as bedrock, hard-packed sand or clay). To ensure that sufficient animals are captured, great care must be taken in the initial site selection. In addition, a considerable amount of debris should be collected at each site. If fewer than 60 animals are found, a replacement site should be selected and sampled. If the second sample still produces less than 60 animals, submit both vials and associated bench sheets, but clearly note the problem and the likely cause on the bench sheets (e.g., hard-packed clay sediments). In those instances where the substrates are not suitable for kick-and-sweep sampling, artificial substrates may be more appropriate. Alternatively, some sort of grab or corer may be used to sample soft substrates in deeper water (e.g., see Klemm et al. 1990).

Standardized counts of approximately 100 animals should provide reliable estimates of the relative proportions of the most common taxa in a sample (Plafkin et al. 1989, Bode et al. 1990). However, the rarer taxonomic groups will be under-represented if too few animals are enumerated (Courtemanch 1996). Since many of the rare taxa include important predators and pollution-sensitive animals, these taxa may be missed if the samples are comprised of substantially less than 100 animals. As a result, we strongly recommend that at least 100 animals are enumerated at each site. Samples with less than 100 animals may prove to be of limited value (Growns et al. 1997).

Once the 100 animals have been picked from the sample, the name of the stream, the riffle number (1, 2 or 3) and the collection date should be recorded on the vial and the enumeration sheet. The same information should be recorded for lakes (i.e., lake name, site number [1-5] and collection date). It is essential that water-proof pencils or china markers are used. Ethanol, isopropanol and water will erase or smear many types of ink or "indelible" markers. Remember that despite all of the work that goes into collecting, sorting and enumerating a sample, the effort is of no value if the origin of the sample is unknown.

Identifying the Animals to Taxonomic Order - It is important to become familiar with the distinguishing characteristics of the various invertebrate groups before attempting to sort and identify the animals. Appendix 1 provides brief

descriptions with the size range, colour, typical movements and an illustration of some of the animals from the more common taxonomic groups. All of these features should be considered before deciding which group a specimen belongs to. In addition, many taxa have distinctive ways of moving through the water. As a result, the way they swim in the tray can help to distinguish different groups that look somewhat similar. For example, mayfly nymphs swim with up-and-down undulations, whereas damselfly nymphs swim with side-to-side undulations.

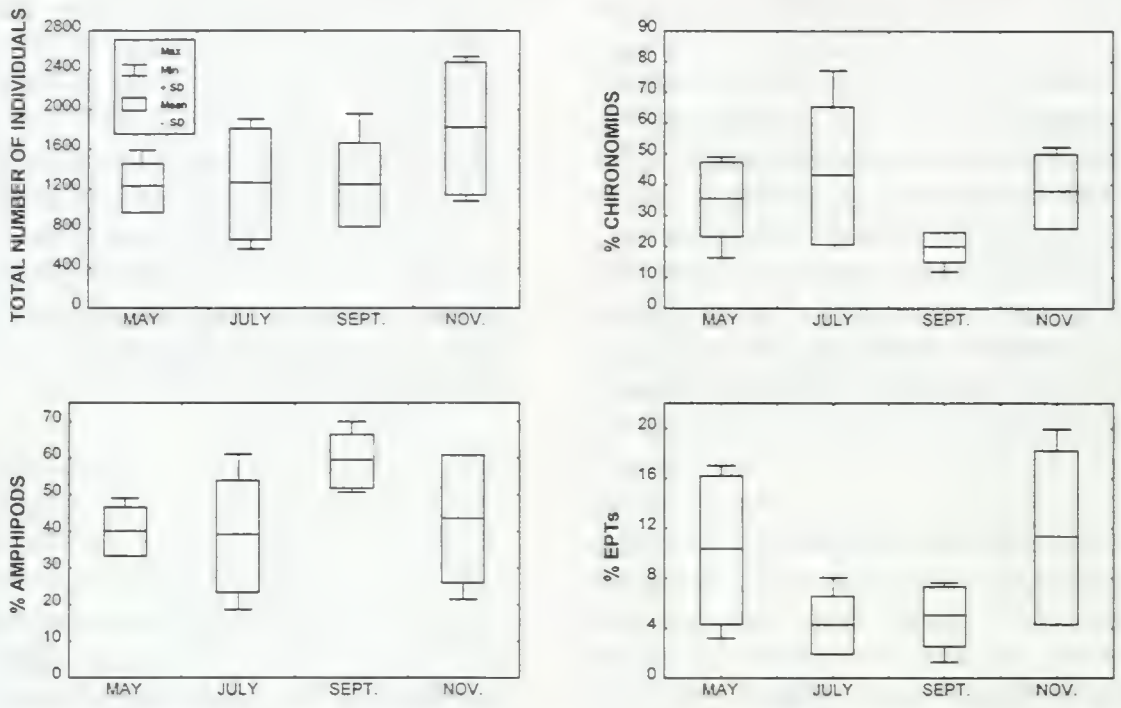
There are many books and manuals that can be used to identify the common macroinvertebrates that are found in streams and lakes. The information in Appendix 1 is provided as a bare minimum. Klemm et al. (1990) include a taxonomic bibliography which cites general references, as well as references for selected taxonomic groups. Reid et al. (1997) provide a shorter list of some of the key references. For those individuals with access to the internet, the Isaak Walton League of America (http://www.iwla.org/iwla/), Save Our Streams program (http: //www.people.virginia.edu/~sos-iwla /Stream-Study/StreamStudyHomePage /StreamStudy.HTML) offers information on a similar rapid bioassessment protocol with taxonomic guides and pictures at the following internet address: http: //www.people.virginia.edu /~sos-iwla /Stream-Study/Key/MacroKeyIntro.HTML

Because the identification of the invertebrate samples is so important, we have proposed that both the bench sheets and the vials containing the 100 animals should be retained and submitted to the rapid bioassessment coordinator (see below). As part of our ground-truthing exercise, we plan to re-examine the samples of 100 animals and produce a second bench sheet. The 2 bench sheets will be compared to identify those taxa that are misidentified and those taxa that are frequently confused. Appendix 1 will be updated accordingly and our choice of biological indices or metrics will be re-assessed in order to accommodate any ambiguous groups.

When to Sample - Many benthic macroinvertebrates moult to winged adults that emerge from the stream or lake to reproduce, disperse and deposit eggs. The timing of emergence and reproduction varies among taxa and among species within taxa. However, both emergence and reproduction are generally affected by water temperature and photoperiod. Because different taxa respond to different combinations of these environmental cues, there is no one best sampling time for all taxa. As a result, samples collected from the same body of water at different times of the year will produce assessments that may be quite different (e.g., see Reid et al. 1995; and see Figure 1). To minimize these sorts of seasonal differences, both test and reference samples should be collected at the same time of the year.

As part of a comparative study of the shallow-water invertebrates in small lakes in south-central Ontario (Reid et al. 1995), we collected samples from 5 sites in Blue Chalk Lake every 6 weeks from May through November (Figure 1). All of the animals in each sample were sorted from

Figure 1: Seasonal Changes in Collections from Blue Chalk Lake



the debris, identified to species or genus, and enumerated. The total number of individuals at a site averaged approximately 1200 in May through September, reaching an average of about 1800 individuals in November. By contrast, the percent amphipods peaked in September when the percent chironomids was lowest. In addition, the proportion of the sample comprised of ephemeropterans (mayflies), plecopterans (stoneflies) and trichopterans (caddis flies) was highest in May and November, but lowest during the summer months when those taxa (i.e., the EPTs) had probably emerged to reproduce. This example of seasonal variation underscores the fact that: (1) different biological indices (e.g., percent amphipods, percent chironomids, percent EPTs) will change in different ways over the ice-free season; and (2), comparisons between test samples and regional reference values (i.e., standards to evaluate a given body of water; see below) should be based on samples collected at approximately the same time of the year.

The 4 seasons each present unique difficulties for stream and lake sampling. Generally winter sampling conditions are adverse at best. Spring and fall sampling may be complicated by exceedingly high (floods) or low (e.g., draw-downs in lakes) water levels, whereas summer conditions may be characterized by low flows, high water temperatures and low dissolved

oxygen concentrations. As a result, test samples collected in the midsummer will probably differ to a greater degree from reference-area samples than test samples collected in the spring or fall.

Although midsummer sampling may be most convenient, many invertebrates have emerged, or are present as very small juveniles during the summer. As a result, it may take considerably more time to remove 100 animals from a sample if most of the animals are quite small. In order to minimize the effects of seasonal variation, we recommend that rapid bioassessments should be completed in the early spring or fall. Periods of high flow associated with spring floods, or periods of low water levels (such as late fall draw-downs in some lakes and reservoirs) should be avoided.

SAMPLE SUBMISSION

Before undertaking a rapid bioassessment, staff with the local Conservation Authority and the regional offices of the Ontario Ministry of Natural Resources and the Ontario Ministry of the Environment should be consulted. If the goal of the assessment is to estimate the biological health of a given body of water relative to other streams or lakes within a particular watershed, then a biologist with the Conservation Authority may serve as the rapid bioassessment coordinator. However, if the goal is to assess the biological impact of a point-source discharge by means of an upstream-downstream comparison (e.g., see Bode et al. 1990) then a biologist with the regional office of the Ministry of Natural Resources or the Ministry of the Environment may serve as the rapid bioassessment coordinator. In essence, the nature of the study will determine the appropriate rapid bioassessment coordinator.

Each rapid bioassessment will produce 3 data sheets and associated vials for a given stream or 5 sheets and associated vials for each lake. Photocopies of the bench sheets should be made by the investigator and saved for future reference. The original bench sheets and the labelled vials should be sent with a covering letter to the appropriate rapid bioassessment coordinator. The covering letter should describe the study area, the reason for the bioassessment and relevant details associated with the field collections (e.g., problems obtaining sufficient animals, identification difficulties, lost samples, etc.). Be sure to include a return address (and a phone or FAX number) in order to receive follow-up information from the rapid bioassessment coordinator.

The coordinator will enter the data from the bench sheets into a simple spreadsheet and verify the accuracy of the computerized data. Ultimately, these data should be stored in a formal database with information on site location and the associated habitat description. The vials of 100 animals should be maintained in a reference collection which will serve as a historical record of the types of animals found at each site. In some instances, the 100 animals may be identified to the level of genus or species in order to characterize sites according to the known pollution tolerances of the

individual species.

Depending on the nature of the study, the data may be used for a variety of different objectives. Initially the bench sheets will be used to evaluate the "biological health" of a particular body of water. To this end, the information on the bench sheets will be used to calculate a variety of biological indices or metrics (see below). These numerical values will be compared to a series of regional reference values to determine whether the sites fall within the normal range of variation, or whether they are atypical. Unusual values may warrant a repeat assessment and subsequent monitoring to ensure no further impairment. Extreme values may prompt a more-detailed biological assessment. If the source of the problem is not evident from the detailed follow-up assessment, water-chemistry testing may be necessary.

Other uses of the data range from quality assurance/quality control (i.e., QA/QC) studies to evaluations of the various biological indices and the biological responses to different stressors. For example, staff at the Dorset Environmental Science Centre are interested in field-testing these protocols, completing QA/QC evaluations and determining which bioassessment indices respond to what stressors. We also hope to evaluate the accuracy of the bench sheets produced by different biologists. We plan to expand this type of assessment to include data submitted by volunteers with less-formal training in the identification of benthic invertebrates. From these comparisons we may find it necessary to expand the taxonomic appendix to further distinguish those groups that are frequently confused. In addition, we hope to use this information to recommend particular biological indices that are sensitive to specific stressors.

DATA SUMMARIZATION

The counts of the individual animals in the different taxonomic groups are the raw data that will be used to evaluate the biological health of a given body of water (e.g., see Appendix 3 or 4). Once these raw data have been entered into a spreadsheet and verified, they will be used to calculate a series of summary biological indices or metrics (e.g., Resh and Jackson 1993, Lenat and Barbour 1994, Metcalfe-Smith 1994). These metrics will be selected from the many indices that are currently used in routine biological assessments (e.g., Plafkin et al. 1989, Barton and Metcalfe-Smith 1992, Gibbons et al. 1993, Norris and Georges 1993; see Table 1). The calculated values for each body of water are then compared to regional reference (or control) values in order to evaluate the sample on a relative scale of biological health. Using this simple comparative approach, the rapid bioassessment coordinator will utilize a series of metrics and determine the relative "health" of the various test samples (e.g., see below). The results of this evaluation will be sent to the individual who submitted the samples.

Types of Biological Indices - There are many different types of biological indices or metrics that have been used by aquatic ecologists (Table 1). Each index has

Table 1: Examples of Different Types of Indices used in Biological Assessments

Type of Index	Example	Explanation
Simple Summaries		
(1) Counts (Richness Measures)		
	(a) Taxonomic Richness	(a) total number of different taxonomic groups found
	(b) Number of Insect Groups	(b) number of taxonomic groups that are insects
(2) Enumerations (Abundance Measures)		
	(a) Total Number of Individuals	(a) total number of invertebrates in sample
	(b) Total Number of EPTs	(b) total number of mayflies, stoneflies and caddisflies
(3) Compositional Indices		
	(a) Percent Amphipods	(a) ratio of number of amphipods to total number of individuals
	(b) Percent Diptera	(b) ratio of number of flies to total number of individuals
(4) Diversity Indices		
	(a) Shannon-Wiener Diversity	(a) evenness of the counts among the taxonomic groups
	(b) Percent Dominants	(b) ratio of most abundant taxon to total number of individuals
Weighted Summaries		
(5) Pollution-Tolerance Indices		
	(a) Trent Biotic Index	(a) pollution-tolerance weighted richness
	(b) Hilsenhoff Biotic Index	(b) pollution-tolerance weighted abundance
Multivariate Summaries - may be used alone or in cluster analyses or ordinations		
(6) Pairwise Similarity Indices		
	(a) Jaccard's Coefficient of Community	(a) degree of taxonomic similarity between two samples
	(b) Percent Similarity	(b) degree of compositional similarity between two samples
(7) Pairwise Distance Indices		
	(a) Euclidean Distance	(a) absolute difference between two samples
	(b) Bray-Curtis Distance	(b) distance complement of percent similarity (1 - PS)
(8) Comparison to a Standard		
	(a) Index of Biotic Integrity	(a) tolerance-weighted sum across a selection of indices
	(b) Percent Model Affinity	(b) relative diffference between a sample and a target
Other Summaries - may include some or all of the above by replacing taxonomic categories		
(9) Trophic or Functional Feeding Groups		
	(a) Number of Predators	(a) classified by feeding habits instead of taxonomy
	(b) Percent Shredders	(b) ratio of shredders to total number of individuals
(10) Groups based on Reproductive Guilds		
	(a) Number of Psammophils	(a) number of taxa reproducing on sandy bottoms
	(b) Percent Phytophils	(b) ratio of individuals that reproduce on plants to total

been constructed to summarize and emphasize particular attributes of the raw data. The most simple summaries involve counts such as the total number of taxonomic groups (i.e., richness measures; type 1 in Table 1) or full enumerations such as the total number of individual organisms in a sample (i.e., abundance measures; type 2 in Table 1). These totals can be standardized to percentages which emphasize relative rather than absolute differences between samples (i.e., compositional indices; type 3 in Table 1). Richness and abundance information can also be combined to produce diversity indices which reflect the relative scatter or variance of the counts among the different taxa (type 4 in Table 1).

In addition to the simple summaries, there is a family of weighted summaries that combine the known pollution tolerances of the different taxa with traditional richness or abundance information (e.g., type 5 in Table 1). As such, pollution tolerances of the different invertebrate taxa are ranked on an arbitrary scale from say, 1 to 10, and the abundances of the different taxa are multiplied by their pollution tolerances, summed and then divided by the total number of individuals to produce a weighted pollution-tolerance value. These weighted indices are commonly employed in situations where the "health" of the invertebrate community is used as a surrogate for a water-quality index (Hilsenhoff 1988, Griffiths 1993, Metcalfe-Smith 1994). One disadvantage of these tolerance-weighted indices is the need to use different tolerance values for different stressors (Klemm et al. 1990). Thus a tolerance value for nutrient enrichment may be quite different from a tolerance value for heavy metals or acidity.

Multivariate summary indices are another group of metrics that are often used with specialized statistical methods to illustrate the biological similarity or difference (distance) among a series of samples (e.g., types 6, 7 and 8 in Table 1). The raw richness and abundance data are the building blocks for these approaches, but these methods focus on comparing multiple pairs of samples (types 6 and 7), or comparing individual samples to an established standard (type 8). Although pairwise indices are occasionally used in upstream-downstream contrasts, or studies of changes through time, the traditional product is a graphical summary in the form of a cluster analysis or ordination (e.g., Jackson 1993, Norris and Georges 1993, Barbour et al. 1996; and see Appendix 6). By contrast, the percent model affinity and multi-metric approaches such as the Index of Biotic Integrity compare individual samples to predetermined reference conditions (Novak and Bode 1992, Karr 1993, Fore et al. 1994, Barton 1996).

Any or all of these 8 different types of biological indices can be recast into a parallel series of metrics by replacing the traditional taxonomic categories with functional feeding groups or even reproductive guilds (types 9 and 10 in Table 1). In this context, the individual taxonomic categories are re-grouped according to feeding or reproductive behaviour. Thus, all of the predatory taxa are grouped together, as are the filterers, the shredders, and so on. Once the raw data have been re-organized, any of the

richness, abundance or other types of indices can be calculated and used like their taxonomic counterparts (e.g., see Cummins and Wilzbach 1985, Resh and Jackson 1993, Lenat and Barbour 1994).

Selecting Biological Indices - Generally an arbitrary subset of indices is used in biological assessments (Plafkin et al. 1989). A single index is rarely used because different stressors can impact members of the benthic community in different ways (Karr 1993, Resh and Jackson 1993). Thus, a single index may suggest an impact, but the type of impact cannot be determined. By contrast, the pattern of "hits and misses" based on several indices often provides a "fingerprint" that implicates particular stressors (Barton and Metcalfe-Smith 1992, Barton 1996, Fore et al. 1996). As a result, a list of potential stressors for a given region and the expected responses of the candidate metrics can assist in choosing among the various indices (Barbour et al. 1996). Unfortunately such a list is generally unavailable.

A number of biological indices are commonly used in bioassessment programs. For example, taxonomic richness, Hilsenhoff's Family Biotic Index, the ratio of scrapers to filtering collectors (a functional feeding group index), the EPT index (i.e., the total number of mayflies, stoneflies and caddis flies), the ratio of EPTs to chironomid abundances, the percent dominants, the community similarity index and the percent shredders are recommended in the US EPA rapid bioassessment protocol (Plafkin et al. 1989). A list of 10 indices is proposed in the BioMAP protocol (Griffiths 1993), although this list involves several different metrics including the total number of individuals, the ratio of chironomids to insects, the percent oligochaetes and the most characteristic taxa.

Comparative studies have provided some guidance for choosing the best biological indices. For example, Barbour et al. (1992) examined the EPA's recommended metrics and advocated the use of taxonomic richness, the EPT index, the Hilsenhoff Index and the percent shredders, but the remaining 4 indices were too variable to be useful. Hannaford and Resh (1995) evaluated the same metrics in a northern California stream and reached similar conclusions, although they retained 2 of the 4 metrics that Barbour et al. (1992) rejected. Similarly, Barton and Metcalfe-Smith (1992) evaluated 8 biological indices and recommended taxonomic richness, a modified Hilsenhoff Index and the percent oligochaetes because of their ability to discriminate sites with known degradation.

Results of our evaluation of 17 indices using rapid bioassessment data from 5 south-central Ontario lakes indicated that the percent amphipods, percent insects and a multivariate metric best distinguished the 5 lakes (see Appendix 6). The repeated success of simple compositional indices based on key taxonomic groups as well as multivariate approaches has probably contributed to the growing popularity of the percent model affinity approach (e.g., see Novak and Bode 1992, Barton 1996). Although multivariate summaries are strongly

advocated (e.g., Jackson 1993, Norris and Georges 1993, Norris 1995, and see Appendix 6), this view is not unanimous among benthic ecologists (e.g., Karr 1993, Fore et al. 1994, Gerritsen 1995) because the multivariate metrics require advanced numerical calculations and they can be more difficult to interpret.

Recommended Indices - Based on the results of comparative studies, a variety of indices from the different types of summaries should be employed to evaluate the biological health of a given body of water. As a result, richness measures, abundances, compositional indices, pollution-tolerance indices and multivariate metrics should all be used (see Table 1). However, final index selection must reflect the type of data generated by the rapid bioassessment protocol. If only 100 animals are counted and they are identified to a relatively coarse taxonomic resolution, then indices that incorporate pollution tolerances and feeding or reproductive guilds may be too coarse to be useful. Preliminary sampling to test the statistical power and general utility of the candidate metrics is advisable (Green 1979). Given these concerns, metrics such as taxonomic richness, percent oligochaetes, percent EPTs, percent midges, percent insects and percent dominants should be used as a minimum (e.g., see Appendix 6). Weighted summaries, multivariate summaries and additional metrics such as functional feeding groups should also be considered, although their use will require supplementary information on pollution tolerances (e.g., Bode et al. 1990, Klemm et al. 1990), feeding behaviour (e.g., Cummins and Wilzbach 1985) and some familiarity with advanced statistical methods (e.g., Norris and Georges 1993).

ASSESSING THE BIOLOGICAL HEALTH OF A BODY OF WATER

These protocols are designed to provide a standardized screening tool to inexpensively estimate the biological health of a given body of water. The methods advocated here were chosen for their simplicity and ease of use. To be consistent with this philosophy we propose a simple comparative approach as a means to evaluate the biological health of a given body of water. This comparative approach requires a set of regional reference data in order to establish a series of benchmarks for the assessment. The regional reference data should be collected at the same time of the year as the samples for the body of water in question. Appropriate regional reference-area information may be available from the rapid bioassessment coordinator. In the example that follows, we will assume that regional reference data are unavailable and thus, are collected at the same time as the test data.

Regional Reference Values - Because there are marked differences in the biological characteristics of streams and lakes in different parts of Ontario, targets for assessing the biological health of a given stream or lake should be based on other bodies of water in the immediate vicinity of the test site. Thus, regional reference sites should be randomly selected from streams or lakes that span the range of acceptable water quality in

the general area of the body of water in question. Here, "acceptable" is a generic term implying that the water quality is normal for that part of the province. In addition, targets based on regional reference sites must be appropriate for the sites in question. For example, a set of pristine, cold headwater streams would not represent attainable targets for streams in a developed, urban setting.

Once a series of reference streams or lakes has been identified, a minimum of 10 bodies of water should be randomly selected and sampled using the standard rapid bioassessment protocol. The resultant bench sheets (i.e., 30 for streams and 50 for lakes) comprise the regional reference data that will be used to establish the normal range of variation for the various biological indices. Critical values (i.e., regional reference values) are easily derived from these regional reference sites. Thus, a test site would be classified as healthy if its values for a variety of different biological indices fell within the normal range of variation defined by the regional reference sites. Values falling outside of the normal range would be classified as atypical or extreme depending on how deviant they are relative to the normal range.

For any given biological index, the normal range of variation can be defined as the range of values that includes 95% of the data from the regional reference sites (e.g., Thompson 1938, Leffler 1978, Kersting 1984, 1988, Kilgour et al. 1998). For any normally distributed variable that is standardized to have a mean of zero and a variance of one (see Figure 2), the area under the normal curve delimited by the mean plus-or-minus 1 standard deviation will enclose approximately 68% of the data points. Similarly, the area under the normal curve represented by the mean plus-or-minus 2 standard deviations encloses 95% of the data points. Thus, the mean plus-or-minus 2 standard deviations provides a logical (albeit arbitrary) definition of the normal range of variation for any normally distributed biological index from a set of regional reference sites. The remaining 5% of the values lying outside this range are unusual or atypical relative to the majority of the values.

Given a normally distributed variable, the mean plus-or-minus 3 standard deviations encloses 99.9% of the data points (Figure 2). Values for a given biological index lying beyond 3 standard deviations from the mean are extreme values representing 1 out of every 1000 randomly selected reference sites. Using this construct, we recognise that 5 out of every 100 reference sites may fall outside of the normal range by chance, and that 1 in 1000 reference sites will be identified as an extreme site. This expected error rate provides a conceptual framework that helps to interpret results based on this regional reference sites approach.

To further simplify the interpretation of results based on regional reference values, test sites falling within the normal range of variation are coloured green to indicate a healthy biological community (Table 3). Test sites that fall between the 95th and 99.9th percentiles are described as atypical relative to the reference sites. These sites would be coloured yellow

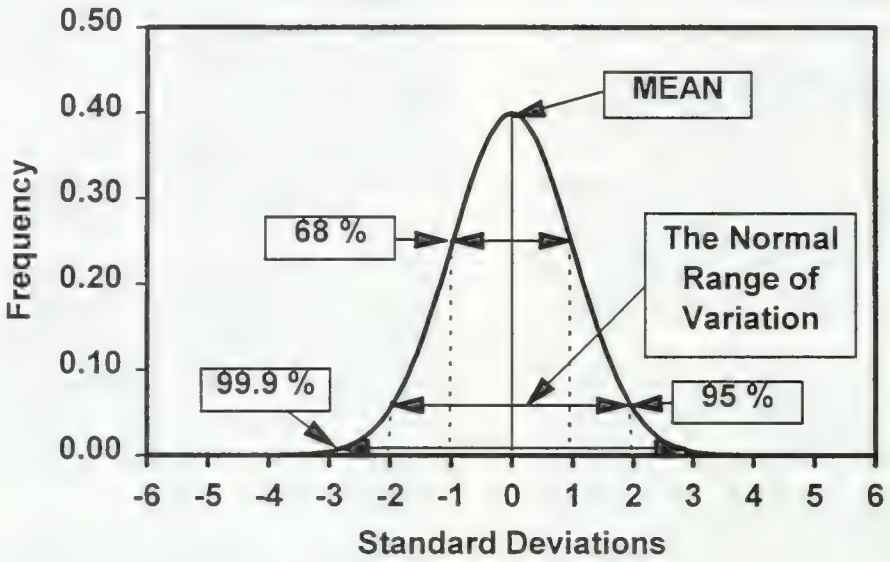


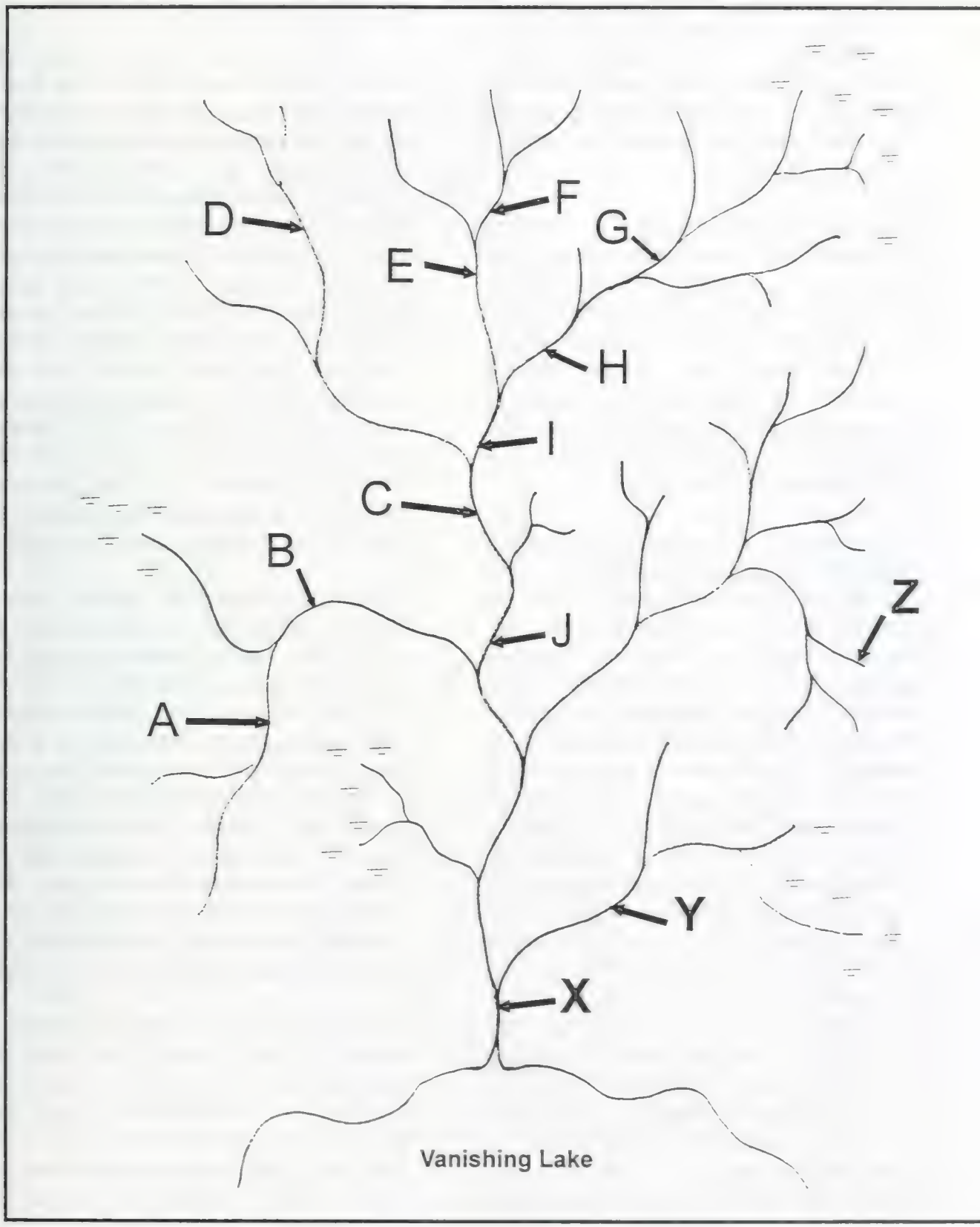
Figure 2: A standardized normal curve illustrating the mean and plus-or-minus one, two and three standard deviations enclosing 68%, 95% and 99.9% of the area under the curve.

suggesting caution since continued monitoring at these sites is warranted. Lastly, those test sites lying outside of the 99.9th percentile are recognised as extreme sites characterized by unusual biological communities. These sites would be coloured red indicating that a detailed biological assessment is recommended in order to more fully understand why these sites are unusual.

The following example using a hypothetical stream drainage network illustrates the basic principles underlying the regional reference values concept (Figure 3). Sites A through J are randomly selected stream segments that were judged to represent background or relatively unimpacted reference conditions for the catchment. Three other stream segments, X, Y and Z, are test sites where the biological health was in question. Triplicate kick-and-sweep samples were collected from each of the reference sites. Each sample was sorted, 100 animals were removed and the corresponding bench sheets were completed.

The raw data from the bench sheets were put into a computerized spreadsheet and verified (Table 2). A subsequent QA/QC

Figure 3: Hypothetical Stream Network with Regional Reference and Test Sites



check revealed that the volunteers on this project often confused the oligochaetes and nematodes. As a result, these 2 groups were combined under the heading of "worms" in the final edited data table. The data were then used to calculate 6 biological indices.

For each of these indices, the resultant 30 values from the reference sites were assumed to characterize the normal range of variation for that metric (Table 2). The distribution of these 30 values was then used in a spreadsheet computer program to estimate a series of percentiles for each index (Table 3). The percentiles in this table are used as the regional reference values.

The 6 biological indices characterize 3 different types of metrics (Table 3). The number of taxonomic groups and the percent EPTs are 2 indices where large values imply a healthy biological community and low values imply reduced health. As a result, the critical percentiles for these metrics are at the low end of the scale (i.e., the 5^{th} and 0.1 percentiles). In the case of the number of taxonomic groups, the 5^{th} percentile (i.e., 13 groups) is the same as the 0.1 percentile. Consequently, an intermediate (or yellow-coloured) range cannot be distinguished with this metric. By contrast, the critical values for the percent EPTs (in this hypothetical example) are 9% (i.e., the 5^{th} percentile) and 7% (i.e., the 0.1 percentile). Thus, test sites with less than 7% EPTs would be extreme values for this catchment, whereas sites with between 7 and 9% EPTs would be identified as atypical. Sites with more than 9% EPTs fall within the normal range of variation for this biological index.

Trends in the percent worms and percent dominants are opposite to the first 2 indices because large values indicate a degraded biological community and small values reflect a healthy community (Table 3). Thus, critical values for these 2 metrics lie at the high end of the scale at the 95^{th} and 99.9^{th} percentiles. Using these 2 indices, healthy sites would support less than 9% worms and the most abundant taxonomic group would comprise less than 45% of the total number of animals. Atypical sites would have between 9 and 10% worms, whereas extreme sites would have more than 10% worms and the dominant taxonomic group would comprise more than 45% of the total number of animals.

The percent diptera and percent insects present a third type of pattern (Table 3). With these indices, both high and low values indicate an unhealthy community, whereas intermediate values imply a healthy community. Here we use a two-tailed distribution such that the normal range lies between the 2.5 and 97.5 percentiles. Atypical values lie outside these 2 percentiles, whereas extreme values fall outside the 0.1 and 99.9 percentiles (actually 0.05 and 99.95 given rounding errors such that 0.1% of the expected values lie outside of this range).

Having specified the regional reference values for the 6 metrics, we can now evaluate the 3 test sites (Table 4). Triplicate samples were collected from each test site at this same time of the year as the regional reference sites. In addition, the bottom (i.e., sediment)

Table 2: Data-Processing Steps for the Regional Reference Site Information

DATA FROM BENCH SHEETS

Taxonomic Group	Hypothetical 10 Reference Area Streams																														Mean Value
	A			B			C			D			E			F			G			H			I			J			
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	
Coelenterata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0
Turbellaria	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0
Nematoda	0	2	1	2	3	1	0	0	0	1	0	2	3	1	0	1	0	1	0	1	0	0	3	0	0	3	1	1	2	3	1.1
Oligochaeta	2	2	1	5	6	4	1	1	3	2	0	2	6	1	1	4	2	1	2	0	0	1	4	3	1	0	1	3	8	5	2.4
Hirudinea	1	0	2	0	0	1	0	2	0	0	1	0	2	0	3	3	5	2	2	3	1	2	2	4	2	1	0	0	0	0	1.3
Isopoda	3	4	2	0	0	0	4	8	2	0	0	0	2	2	1	6	8	5	5	3	5	3	7	8	2	0	0	5	11	9	3.5
Amphipoda	0	0	0	15	11	8	0	0	0	6	3	3	0	0	3	0	0	0	0	4	0	0	0	0	0	1	0	0	0	1	1.8
Decapoda	1	0	1	0	0	2	3	1	3	2	0	1	0	2	1	0	1	1	0	0	1	1	0	1	1	2	0	2	1	0	0.9
Hydracarina	0	2	3	2	0	4	6	2	1	5	8	3	2	0	0	3	6	2	0	0	1	3	1	6	3	4	6	6	4	0	2.8
Ephemeroptera	12	15	16	6	8	11	19	13	7	12	21	14	6	16	7	8	4	11	14	18	16	11	3	4	11	7	13	21	17	28	12.3
Anisoptera	0	1	0	0	1	0	2	0	2	0	1	0	0	1	0	1	0	2	0	0	2	0	1	0	0	0	1	1	0	1	0.6
Zygoptera	2	1	1	0	1	2	2	2	1	0	1	0	2	3	1	0	1	0	0	0	0	1	0	1	1	0	0	0	0	1	0.8
Plecoptera	11	7	9	3	3	8	17	8	6	15	17	10	2	16	14	9	6	13	14	17	21	9	4	6	15	12	19	5	7	11	10.5
Hemiptera	2	1	4	0	8	5	0	0	2	0	4	0	0	3	2	1	0	1	0	1	1	4	1	0	4	9	3	3	1	1	2.0
Megaloptera	0	1	3	0	1	2	2	4	0	3	5	2	0	3	2	0	0	3	1	2	3	1	0	0	3	3	5	1	1	0	1.7
Trichoptera	10	7	5	1	0	5	13	12	16	3	17	2	0	3	9	2	2	11	7	11	14	8	0	2	12	8	4	3	6	0	6.4
Lepidoptera	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0
Coleoptera	4	5	2	3	3	0	2	0	1	3	4	6	5	0	3	3	0	6	2	0	6	2	3	0	2	0	0	5	3	0	2.4
Chironomidae	32	41	29	26	19	23	24	31	28	35	22	36	34	27	27	39	27	44	23	17	18	19	36	22	12	26	19	34	22	27	27.3
Tabanidae	0	1	0	6	1	0	2	0	1	0	0	0	2	0	0	3	0	0	2	1	0	1	3	0	0	0	0	1	0	0	0.8
Culicidae	0	0	0	1	0	0	1	0	0	1	0	0	0	0	0	2	0	0	0	0	0	0	2	0	0	0	1	0	1	1	0.3
Ceratopogonidae	2	3	2	4	0	6	0	3	2	4	3	6	1	0	0	1	6	2	2	3	2	2	1	4	2	6	3	5	4	9	2.9
Tipulidae	0	0	0	2	7	3	0	0	0	1	0	0	0	0	0	3	0	0	0	0	0	1	3	0	0	0	0	0	1	0	0.7
Simuliidae	0	0	0	0	0	0	3	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0.2
Gastropoda	14	8	11	18	24	15	12	7	18	13	5	7	21	10	13	13	18	4	11	12	12	23	22	26	9	6	11	1	6	3	12.4
Pelecypoda	7	0	6	2	4	6	6	7	2	4	11	10	7	6	11	5	9	1	14	8	3	11	4	9	11	15	9	0	1	1	6.3
Total Count	103	101	98	96	100	106	119	101	95	110	125	104	95	94	98	107	95	110	99	101	106	104	100	96	92	103	96	97	96	101	101.6

EDITED DATA

Combined Groups	A			B			C			D			E			F			G			H			I			J			Mean Value
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	
Worms	2	4	2	7	9	5	1	1	3	3	0	4	9	2	1	5	2	2	2	1	0	1	7	3	1	3	2	4	10	8	3.5
Leeches	1	0	2	0	0	1	0	2	0	0	1	0	2	0	3	3	5	2	2	3	1	2	2	4	2	1	0	0	0	0	1.3
Sowbugs	3	4	2	0	0	0	4	8	2	0	0	0	2	2	1	6	8	5	5	3	5	3	7	8	2	0	0	5	11	9	3.5
Scuds	0	0	0	15	11	8	0	0	0	6	3	3	0	0	3	0	0	0	0	4	0	0	0	0	0	1	0	0	0	1	1.8
Crayfish	1	0	1	0	0	2	3	1	3	2	0	1	0	2	1	0	1	1	0	0	1	1	0	1	1	2	0	2	1	0	0.9
Mites	0	2	3	2	0	4	6	2	1	5	8	3	2	0	0	3	6	2	0	0	1	3	1	6	3	4	6	6	4	0	2.8
Mayflies	12	15	16	6	8	11	19	13	7	12	21	14	6	16	7	8	4	11	14	18	16	11	3	4	11	7	13	21	17	28	12.3
Dragonflies	0	1	0	0	1	0	2	0	2	0	1	0	0	1	0	1	0	2	0	0	2	0	1	0	0	0	1	1	0	1	0.6
Damselflies	2	1	1	0	1	2	2	2	1	0	1	0	2	3	1	0	1	0	0	0	0	1	0	1	1	0	0	0	0	1	0.8
Stoneflies	11	7	9	3	3	8	17	8	6	15	17	10	2	16	14	9	6	13	14	17	21	9	4	6	15	12	19	5	7	11	10.5
Bugs	2	1	4	0	8	5	0	0	2	0	4	0	0	3	2	1	0	1	0	1	1	4	1	0	4	9	3	3	1	1	2.0
Alderflies	0	1	3	0	1	2	2	4	0	3	5	2	0	3	2	0	0	3	1	2	3	1	0	0	3	3	5	1	1	0	1.7
Caddisflies	10	7	5	1	0	5	13	12	16	3	17	2	0	3	9	2	2	11	7	11	14	8	0	2	12	8	4	3	6	0	6.4
Beetles	4	5	2	3	3	0	2	0	1	3	4	6	5	0	3	3	0	6	2	0	6	2	3	0	2	0	0	5	3	0	2.4
Diptera	34	45	31	39	27	32	30	34	31	41	26	42	37	27	27	48	33	46	27	21	20	24	45	26	15	32	23	40	28	37	32.3
Snails	14	8	11	18	24	15	12	7	18	13	5	7	21	10	13	13	18	4	11	12	12	23	22	26	9	6	11	1	6	3	12.4
Clams	7	0	6	2	4	6	6	7	2	4	11	10	7	6	11	5	9	1	14	8	3	11	4	9	11	15	9	0	1	1	6.3
Total Count	103	101	98	96	100	106	119	101	95	110	124	104	95	94	98	107	95	110	99	101	106	104	100	96	92	103	96	97	96	101	101.6

EXAMPLE BIOLOGICAL INDICES

Metric	A			B			C			D			E			F			G			H			I			J			Mean Value
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	
Number of Groups	14	16	17	15	15	17	17	14	16	16	17	14	14	14	15	18	13	17	13	14	15	19	17	13	17	14	14	16	17	14	15.4
EPTs (%)	32	29	31	10	11	23	41	33	31	27	44	25	8	37	31	18	13	32	35	46	48	27	7	13	41	26	38	30	31	39	28.5
Worms (%)	2	4	2	7	9	5	1	1	3	3	0	4	9	2	1	5	2	2	2	1	0	1	7	3	1	3	2	4	10	8	3.5
Dominants (%)	33	45	32	41	27	30	25	34	33	37	21	40	39	29	28	45	35	42	27	21	20	23	45	27	16	31	24	41	29	37	31.8
Diptera (%)	33	45	32	41	27	30	25	34	33	37	21	40	39	29	28	45	35	42	27	21	19	23	45	27	16	31	24	41	29	37	31.8
Insects (%)	73	82	72	54	52	61	73	72	69	70	77	73	55	77	66	67	48	85	66	69	78	58	57	41	68	69	71	81	66	78	67.7

Table 3: Example Regional Reference Values for the Metrics from the Hypothetical data

Metric	Percentiles using the 30 Samples from the Reference-Area Streams												
	0.1	1	2.5	5	10	25	50	75	90	95	97.5	99	99.9
Number of Groups	13	13	13	13	14	14	15	17	17	18	18	19	19
EPTs (%)	7	7	8	9	11	23	31	37	42	45	46	47	48
Worms (%)	0	0	0	0	1	1	2	5	8	9	10	10	10
Dominants (%)	16	17	19	20	21	27	31	39	42	45	45	45	45
Diptera (%)	16	17	18	20	21	27	31	39	42	45	45	45	45
Insects (%)	41	43	46	50	54	62	69	73	79	82	83	84	84

Legend:	Colour and Interpretation	Action to be Taken
Green	Normal Range of Variation - Unimpacted	No further action necessary
Yellow	Atypical Value beyond the Normal Range	Repeat assessment and continue to monitor
Red	Extreme Value - Potentially Impacted	Repeat assessment - follow-up suggested

characteristics of the test sites were similar to the regional reference sites in order to ensure that the observed differences were not due to simple differences in habitats. The data on the bench sheets were treated the same way as the original reference-site data to produce a sub-table of edited data. Because we expect some degree of variation within a given test site, the 3 replicate samples at each test site were averaged to produce a mean abundance for each taxonomic group. These mean values were used to calculate the 6 biological metrics for each site and these values were evaluated with respect to the regional reference values (Table 3).

For site X, 5 of the 6 metrics fell outside of the normal range of variation (Table 4). The number of taxonomic groups and percent EPTs were 9 and 1, respectively. These values were substantially less than the 0.1 percentile that separated extreme from atypical sites. Similarly, site X supported 37% worms which was much larger than the critical value of 10%. By contrast, the percent dominants fell within the normal range, but the percent diptera and percent insects were extreme values. This result emphasizes that each metric provides a different image of the biological community at a given site. Consequently, each of the different indices may classify a site somewhat differently. This does not mean that some indices are right and others are wrong. What it does mean is that different stressors affect different taxa in the benthic community (e.g., nutrient enrichment often leads to a benthic community comprised almost entirely of oligochaetes [worms]). Thus the impacts of different stressors will be realized by different patterns of passes and failures for the various indices. Ultimately, we hope to use these patterns of passes and failures as a diagnostic tool to predict what type of stressor is affecting the biological community at a given test site. Unfortunately this diagnostic information is not available for most stressors.

A different pattern was evident in the assessment of site Y (Table 4). Four of the indices fell within the normal range based on the regional reference sites (Table 3). That is, the percent EPTs, percent dominants, percent diptera and percent insects were all typical of the regional reference sites. However, the total number of taxonomic groups (13) was lower than normal and fell within the atypical (yellow) range. In addition, the percent worms was greater than normal, lying in the extreme (red) range.

The third test site, Z, was somewhat intermediate with respect to X and Y (Table 4). Three indices fell within the normal range of variation (i.e., the percent EPTs, percent worms and percent dominants). The number of taxonomic groups was quite low, lying below the "extreme" regional reference value. In addition, the percent diptera value was also much less than the "extreme" threshold, whereas the percent insects was much larger than the normal range.

Interpreting Results based on Regional Reference Values - From this comparison, all 3 of the test sites are unusual to some degree. Site X was classified as an extreme site for 5 of the 6 indices. Site Z was extreme for 3 indices.

Table 4: Example Calculations for the Data from the 3 Test Sites

DATA FROM BENCH SHEETS

Taxonomic Group	Hypothetical "Test" Streams								
	X			Y			Z		
	1	2	3	1	2	3	1	2	3
Coelenterata	0	0	0	0	0	0	0	0	0
Turbellaria	0	0	0	0	0	0	0	0	0
Nematoda	0	0	1	3	0	0	0	0	1
Oligochaeta	21	53	35	24	11	3	0	0	0
Hirudinea	0	0	0	1	0	0	0	0	0
Isopoda	0	0	0	1	0	0	0	0	0
Amphipoda	3	1	11	1	0	1	0	0	0
Decapoda	0	0	0	0	0	1	0	0	0
Hydracarina	0	0	0	0	0	1	0	1	1
Ephemeroptera	0	0	0	1	2	0	34	28	31
Anisoptera	0	1	0	0	0	0	0	0	0
Zygoptera	0	0	0	0	0	0	0	0	0
Plecoptera	0	0	0	2	0	0	12	4	11
Hemiptera	3	1	0	0	0	0	0	0	0
Megaloptera	0	0	0	0	0	0	1	3	1
Trichoptera	1	2	0	13	26	32	20	19	11
Lepidoptera	0	0	0	0	0	0	0	0	0
Coleoptera	0	1	1	10	12	13	35	19	33
Chironomidae	10	14	11	33	45	43	2	10	11
Tabanidae	2	1	1	0	0	0	0	0	0
Culicidae	0	0	3	3	5	0	0	3	1
Ceratopogonidae	0	0	0	0	0	0	3	3	0
Tipulidae	0	0	0	0	0	0	1	0	1
Simulidae	0	0	0	0	0	0	0	0	0
Gastropoda	40	22	30	3	8	3	0	2	1
Pelecypoda	13	7	5	3	5	5	1	1	1
Total Count	93	103	98	98	114	102	109	93	104

EDITED DATA

Combined Groups	Hypothetical "Test" Streams								
	X			Y			Z		
	1	2	3	1	2	3	1	2	3
Worms	21	53	35	24	11	3	0	0	0
Leeches	0	0	0	1	0	0	0	0	0
Sowbugs	0	0	0	1	0	0	0	0	0
Scuds	3	1	11	1	0	1	0	0	0
Crayfish	0	0	0	0	0	1	0	0	0
Mites	0	0	0	0	0	1	0	1	1
Mayflies	0	0	0	1	2	0	34	28	31
Dragonflies	0	1	0	0	0	0	0	0	0
Damselflies	0	0	0	0	0	0	0	0	0
Stoneflies	0	0	0	2	0	0	12	4	11
Bugs	3	1	0	0	0	0	0	0	0
Alderflies	0	0	0	0	0	0	1	3	1
Caddisflies	1	2	0	13	26	32	20	19	11
Beetles	0	1	1	10	12	13	35	19	33
Diptera	12	15	15	36	50	43	6	16	13
Snails	40	22	30	3	8	3	0	2	1
Clams	13	7	5	3	5	5	1	1	1
Total Count	93	103	97	95	114	102	109	93	103

SUMMARIZED DATA

Combined Groups	Hypothetical "Test" Streams		
	X	Y	Z
	Mean	Mean	Mean
Worms	36.3	12.7	0.0
Leeches	0.0	0.3	0.0
Sowbugs	0.0	0.3	0.0
Scuds	5.0	0.7	0.0
Crayfish	0.0	0.3	0.0
Mites	0.0	0.3	0.7
Mayflies	0.0	1.0	31.0
Dragonflies	0.3	0.0	0.0
Damselflies	0.0	0.0	0.0
Stoneflies	0.0	0.7	9.0
Bugs	1.3	0.0	0.0
Alderflies	0.0	0.0	1.7
Caddisflies	1.0	23.7	16.7
Beetles	0.7	11.7	29.0
Diptera	14.0	43.0	11.7
Snails	30.7	4.7	1.0
Clams	8.3	4.3	1.0
Total Count	97.7	103.7	101.7

SELECTED METRICS AND SITE STATUS

Metric	X	Y	Z
Number of Groups	9	13	9
EPTs (%)	1	24	56
Worms (%)	37	12	0
Dominants (%)	37	41	30
Diptera (%)	14	41	11
Insects (%)	18	77	97

whereas site Y had 1 extreme value and 1 atypical value. As a result, we might be tempted to rank the 3 test sites in order of biological health from site X as poor, site Z also poor but better than X, and site Y as better than X and Z, but not within the normal range. However, we caution against this simplistic approach because each biological index responds to different stressors in different ways. Moreover, some metrics may be more sensitive to certain stressors than others. Thus, a pass or fail on 1 metric is not equal to a pass or fail on a different index. As a result, the number of passes or fails should not be used to compare sites.

The status of a site based on each index provides insights into the potential stressors affecting the benthic community at that site (e.g., a very high percentage of oligochaetes and virtually no other taxa might implicate nutrient enrichment as the most important stressor at a site). A cumulative index contrasting the number of extreme, atypical and normal metrics hides this important information.

Returning to the specifics of the hypothetical data, the benthic community at site X was constructed to resemble a community from a site impacted by urban development. The benthic community at site Y was chosen to characterize a site that was modestly impacted by agricultural runoff. By contrast, the macroinvertebrate community at site Z was chosen to represent a cold headwater stream. Thus, the benthic community at site Z was truly atypical of the regional reference sites, but not because of degradation due to human activities. In this case, the regional reference sites were inappropriate for site Z because that site should have been assessed with respect to a set of reference sites that were cold headwater streams.

These results lead to 2 conclusions: (1) the regional reference sites must be appropriate for the test sites; and (2), a cumulative total based on the number of passes or failures will make it almost impossible to diagnose the cause of (or reason for) the observed impairment. Thus, a summary table (e.g., see Table 4) with the various metrics and associated colour codes should prove to be more informative than simply ranking test sites or constructing some form of "degradation index" that ranges from 0 to 10.

Unfortunately region-specific diagnostic charts outlining the stressors that affect the different biological indices are not available. Until sufficient rapid bioassessment data are collected to construct these diagnostic charts, we believe that rapid bioassessments should be evaluated using the regional reference value approach. Sites that are characterized by metrics lying within the normal range of variation require no further investigation (i.e., they are healthy). Sites that are classified as atypical should be re-sampled and monitored on a regular basis to watch for changes in their status. By contrast, sites that are identified as extremes are candidates for more traditional detailed benthic invertebrate assessments and water quality testing. However, the final interpretation of the significance of the results rests with the rapid bioassessment coordinator.

ACKNOWLEDGEMENTS

This report outlines protocols currently used for the rapid bioassessment of streams and lakes by the Aquatic Sciences Section of the Ontario Ministry of the Environment. These protocols were developed through collaboration with Ron Ingram, of the Dorset Environmental Science Centre, Les Stanfield and Mike Jones of the Ontario Ministry of Natural Resources, Linda Corkum at the University of Windsor, and Dave Barton at the University of Waterloo. Don Jackson at the University of Toronto and Bruce Kilgour at the University of Waterloo also suggested solutions to various problems that we encountered. John Gunn and Ed Snucins with the Ontario Ministry of Natural Resources field tested the protocols described in the first edition of this report. Numerous staff and students also assisted with field collections and sample sorting. We thank all of these individuals and all of the rapid bioassessment volunteers for their contributions. We also thank Susan Dreier, Bruce Hawkins, Peter Kauss and Peter Dillon for comments on the draft copy of this report.

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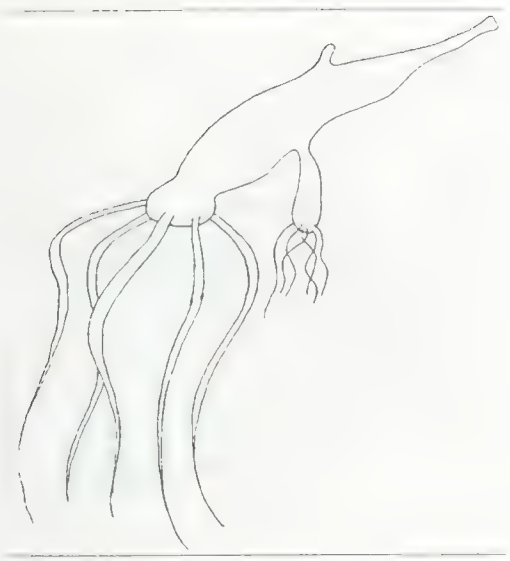
APPENDIX 1

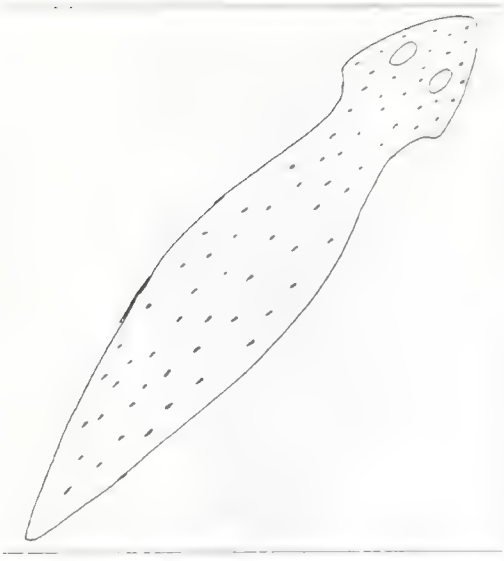
GUIDE TO DISTINGUISHING CHARACTERISTICS

OF COMMON AQUATIC INVERTEBRATES



Coelenterata (Hydra)

- inconspicuous, 2-25 mm long
- colour varies but often clear to whitish
- tubular shape with tentacles (little octopus)
- usually attached to an object



Turbellaria (unsegmented flatworms)

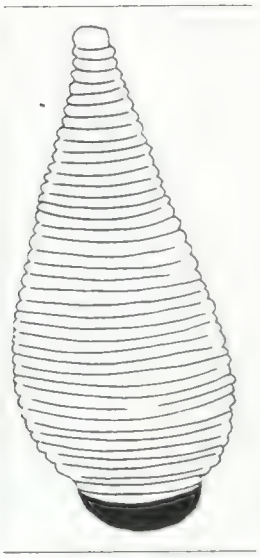
- 5-30 mm long, greyish-brown or colourless
- very flat 'worms', heads with eyespots
- non-swimmers
- creep slowly on bottom of sorting tray

Nematoda (transparent worms)

- usually less than 1 cm long
- head usually tapered, tail pointed
- not segmented, frequently clear
- detected by their rapid, whip-like movements

Oligochaeta (aquatic worms)

- 1 to 30 mm long, often pinkish
- look like earthworms
- segmented bodies, pointed head and tail
- crawl along bottom of tray



Hirudinea (leeches)

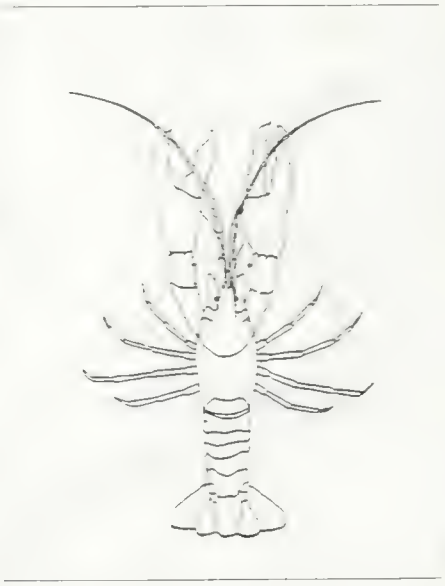
- 5mm-30 cm long
- colour varies often brown or black
- some green on top, orange on bottom
- suckers at both ends, move by inching along

Isopoda (sow bugs)

- 5-20 mm long; brown, black, reddish or yellow
- very flat, segmented crustacean with many legs
- often associated with plants, moss, etc.
- crawl slowly in sorting tray

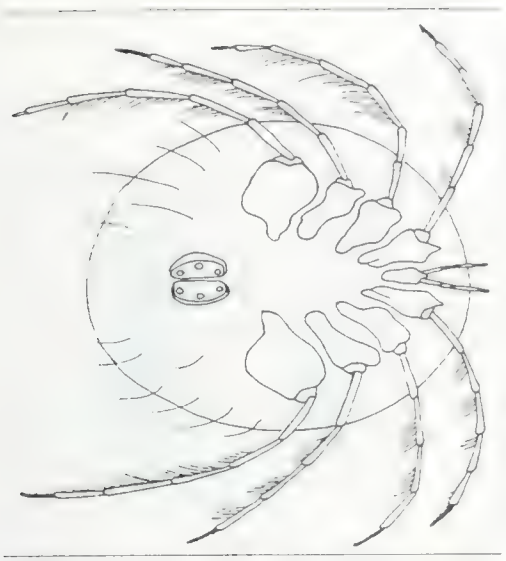
Amphipoda (scuds, sideswimmers)

- 5-20 mm long, bluish grey, brown, green, red
- tiny, curled up shrimp; half circle shape
- long antennae and many legs, fast swimmers
- appear to be lying on their side
- catch with eyedropper



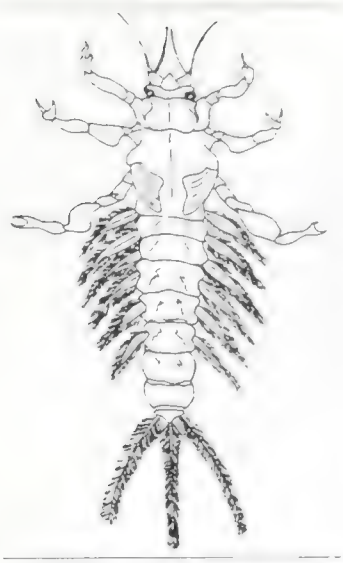
Decapoda (crayfish, shrimp)

- 1 to 15 cm long, often green, brown, blue
- look like small lobsters
- large claws, hard-shelled, eyes on stalks
- walk without turning on side
- swim quickly backwards to escape



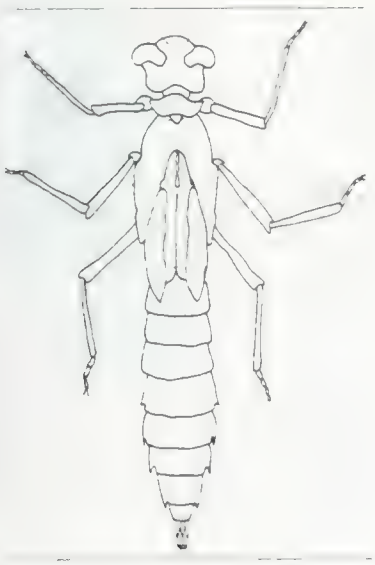
Hydracarina (mites)

- 0.4-3 mm bright red, green, blue or brown
- look like small spiders, eight legs
- bodies soft and round or oval shaped
- uncoordinated, scrambling swimming motion
- *magnified bottom view shown*



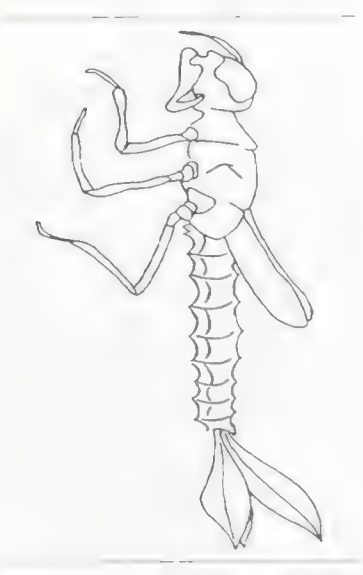
Ephemeroptera (mayflies)

- 3-28 mm long not counting the 3-spined tail
- brown or brownish black, many shapes
- large body and head, 6 legs that stick out
- feather like gills under abdomen
- swim up and down in 's' pattern



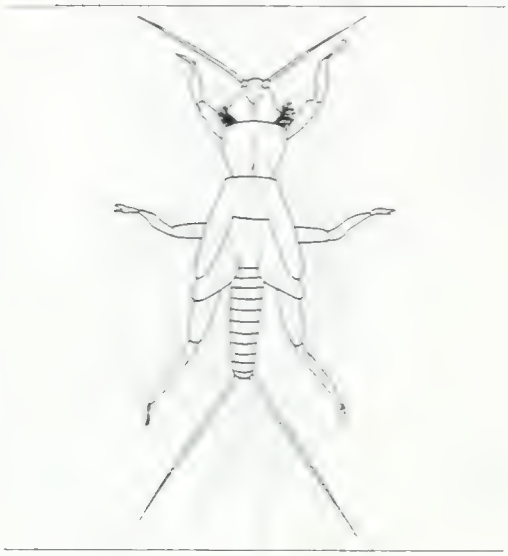
Odonata-Anisoptera (dragonflies)

- 15-45 mm, green to greenish brown
- larger than mayflies with no tail
- large eyes, protruding lip, variable shapes
- often flat, legs stick out sides, slow moving



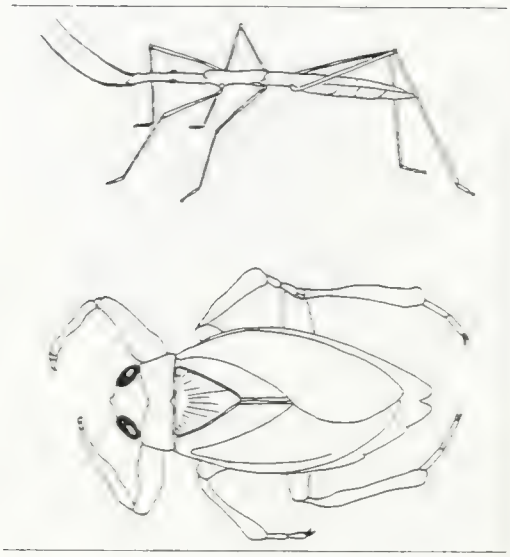
Odonata-Zygoptera (damselflies)

- 10 to 22 mm long, green to greenish brown
- bodies more tubular, thinner than dragonflies
- have a tail with 3 gills unlike dragonflies
- three pairs of legs that stick out at the sides.
- more active than dragonflies



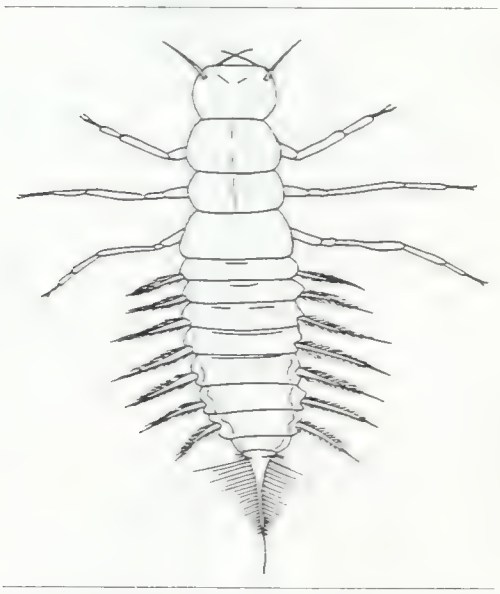
Plecoptera (stonefly)

- 6-50mm, yellowish, brown or blackish
- resemble mayflies but with 2 tails
- sometimes feather like gills under body
- flattened with legs out at sides, slow moving



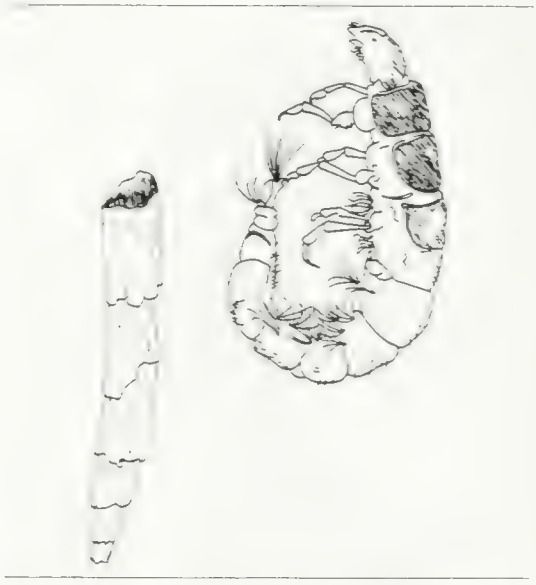
Hemiptera (true bugs)

- 15-40 mm, black or brownish
- legs often long, stick out from sides
- often two pair of folded wings (not hard)
- variable shapes, soft bodies



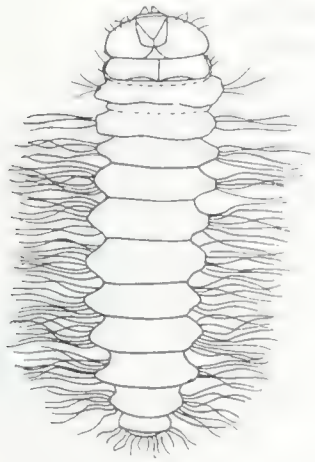
Megaloptera (alder flies, hellgrammite)

- 25-90 mm long, brown
- large hairy crawlers resembling centipedes
- hairs are gills on first few abdominal segments
- carnivorous predators



Trichoptera (caddis flies)

- 2 - 50 mm long, catterpillar shape
- head and thorax (3 segments) dark and hard
- body soft and cream coloured,
- 3 pairs of legs near front, slow movers
- often in 'house' of sticks, stones or sand

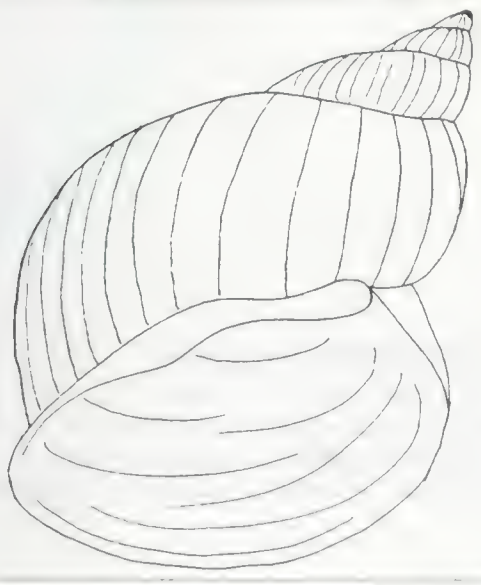


Lepidoptera (aquatic caterpillar)

- 10 - 25 mm, yellow, white or brown
- resembles caterpillar with short legs along body
- no dark segment near head like caddis flies
- crawl on vegetation or bottom of tray

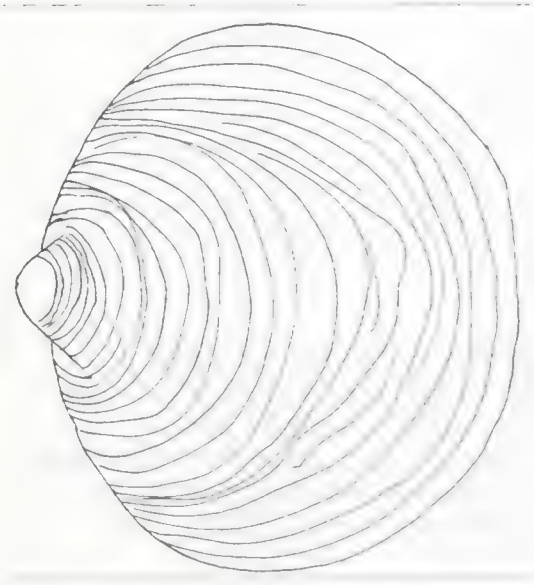
Coleoptera (riffle [water] beetles)

- 2 - 20 mm, brownish (adults illustrated)
- most have 3 pairs of legs near the head
- larva may look like caddis flies but body is hard
- not more than 1 pair of small legs on abdomen



Gastropoda (snails, limpets)

- 2 - 70 mm, gray, brown or black
- hard shelled, spiral shape
- various sizes shapes and colours
- often floating in tray
- *do not count empty shells*

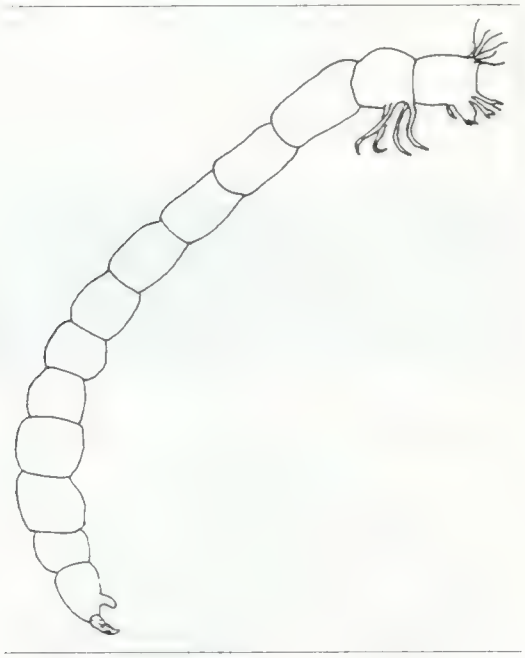


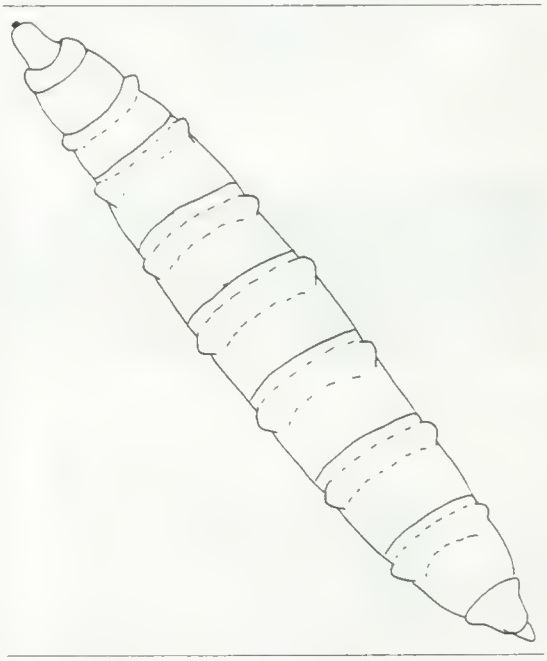
Pelecypoda (clams, mussels)

- 2 - 250 mm, white, yellow, brown, green, black
- hard oval shell hinged in two halves
- found in bottom of tray in sand or gravel
- *do not count empty (dead) shells*

Diptera (flies, mosquitoes, midges)

- divided into 6 main groups that are illustrated on the NEXT TWO PAGES
- adults only have 2 wings and disperse by flying to other bodies of water
- generally represented by larvae and pupae (not adults) that are found in the sediments
- larvae are distinguished by their short, maggot-like bodies with no eyes or jointed legs
- some dipteran larvae and pupae have "bumps" or prolegs on the abdomen (like a caterpillar)
- quite variable in size, shape and colour



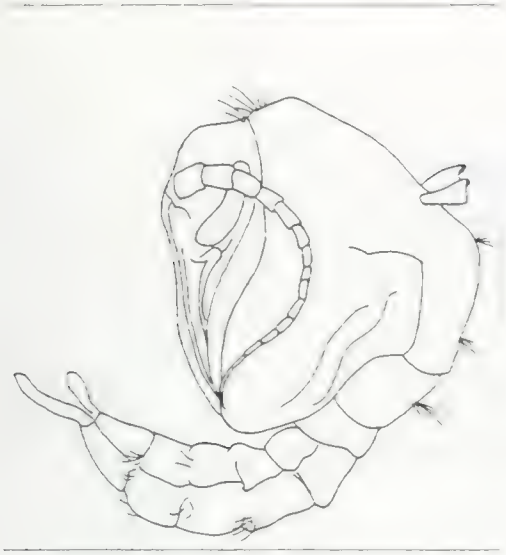


Chironomidae (midges)

- 2 - 30 mm long, red, white or yellowish
- characteristic shape like letter "J"
- may be in a tube made of fine dirt particles
- slowly creep along bottom of tray
- often caught in surface film

Tabanidae (horseflies)

- 15 - 40 mm, white or yellowish
- pointed at both ends, leathery texture
- segmented, straight or slightly curved
- seven pairs of small bumps along abdomen

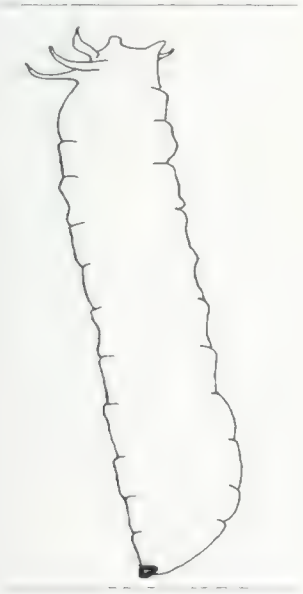


Culicidae (mosquito larva & pupa)

- 3-15 mm, brown, various shapes
- characteristic thickened body near head
- segmented body, curved at one end
- twitch in water when disturbed

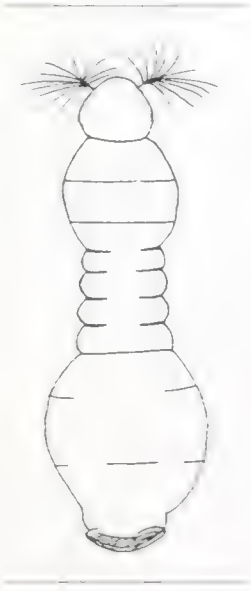
Ceratopogonidae(no-see-um, biting midges)

- 3-13mm, whitish somewhat transparent
- very slender, pointed at both ends, segmented
- remain stiff when picked up with forceps
- move in a snake-like whipping fashion



Tipulidae (crane fly larvae & pupae)

- 10-50 mm, white, yellowish or brown
- leathery, semented, plump "maggot"
- pointed at both ends
- one end with finger-like projections or lobes



Simuliidae (black fly larvae & pupae)

- 3 - 15 mm, brown or greyish clour
- like flattened maggot with one end 1/3 fatter
- move with looping (inch-worm) movements
- often found in large numbers

APPENDIX 2

BENTHIC ENUMERATION SHEET

Lake or Stream Name: _____ Stream Code: _____ Site Code: _____ Date: ____ • ____ • ____ Crew: _____

Technique used: _____ Replicate number: _____ Number of Bottles: _____ Water Depth: _____ Slope (in): _____

Substrates present: Clay: ____ Silt: ____ Sand: ____ Gravel: ____ Cobble: ____ Boulder: ____ Bedrock: ____

COELENTERATA
(hydra)

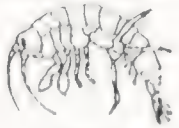
TURBELLARIA
(flatworm)

NEMATODA
(unsegmented worm)

OLIGOCHAETA
(segmented worm)

HIRUDINEA
(leech)

ISOPODA
(sow bug)



AMPHIPODA
(scud)

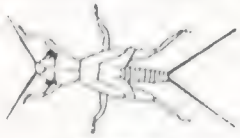
DECAPODA
(crayfish)

HYDRACARINA
(mite)

EPHEMEROPTERA
(mayfly)

ODONATA - ANISOPTERA
(dragonfly)

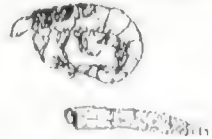
ODONATA - ZYGOPTERA
(damselfly)



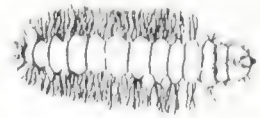
PLECOPTERA
(stonefly)

HEMIPTERA
(back swimmer)

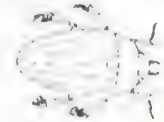
MEGALOPTERA
(fishfly)



TRICHOPTERA
(caddisfly)



LEPIDOPTERA
(aquatic caterpillar)



COLEOPTERA
(riffle beetle)

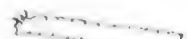
Chironomidae
(midge larva)

Tabanidae
(horsefly)

Culicidae
(mosquito)

DIPTERA

Ceratopogonidae
(no-see-um)



Tipulidae
(crane fly)

Simuliidae
(blackfly)

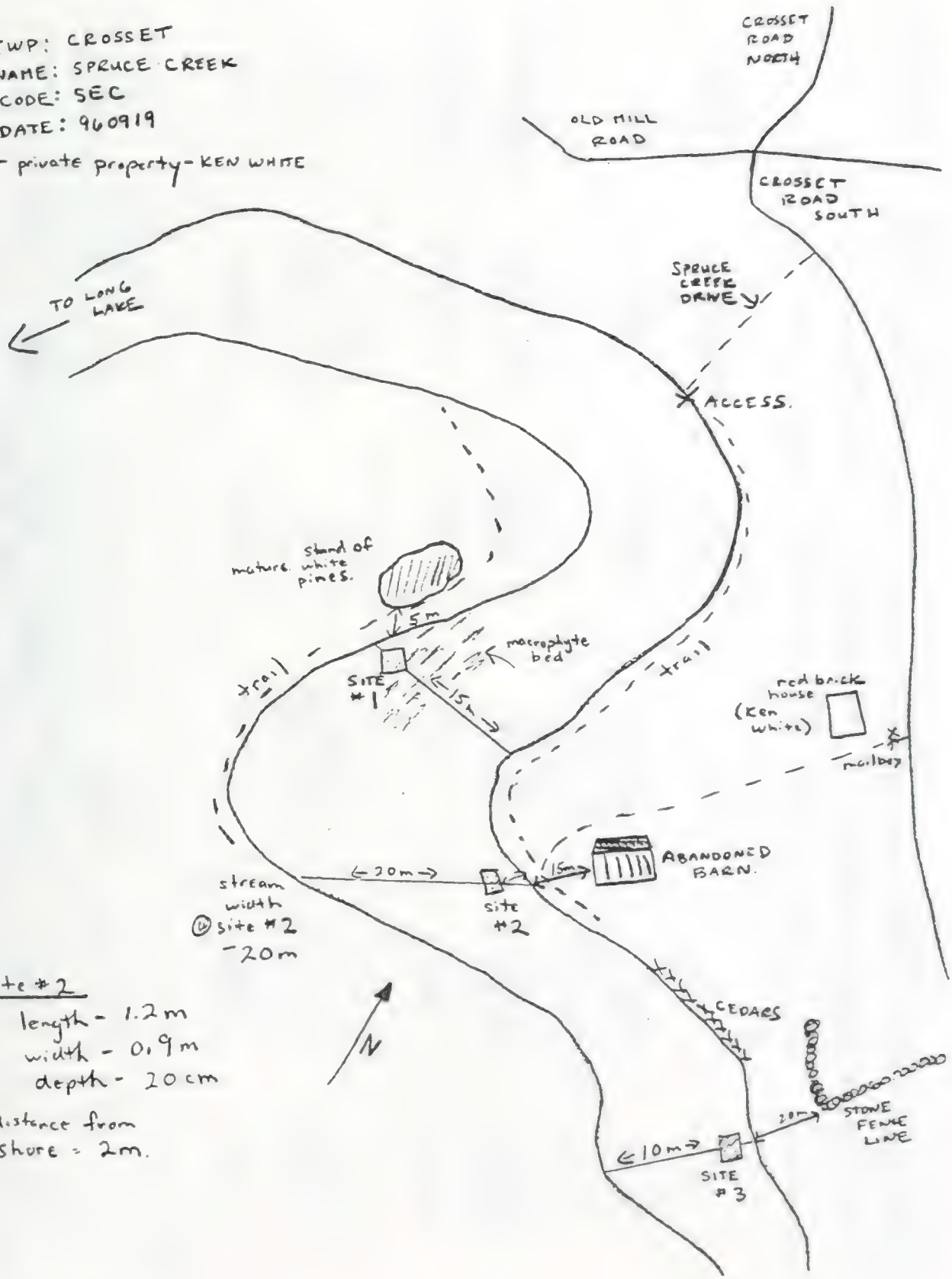
GASTROPODA
(snail)

PELECYPODA
(clam)

APPENDIX 3

EXAMPLE 1: COMPLETED STREAM SURVEY SHEET

TWP: CROSSET
NAME: SPRUCE CREEK
CODE: SEC
DATE: 960919
- private property - KEN WHITE



Site #2

length - 1.2m
width - 0.9m
depth - 20cm

distance from shore = 2m.

Lake or Stream Name: SPRUCE CREEK Stream Code: SEC Site Code: 2 Date: 96 · 09 · 19 Crew: SMITH, D. LAWRENCE

Technique used: KICK + SWEEP Replicate number: _____ Number of Bottles: 1 Water Depth: _____ Slope (in): _____

Substrates present: Clay: ____ Silt: 15 Sand: 25 Gravel: 40 Cobble: 20 Boulder: ____ Bedrock: _____

COELENTERATA (hydra)	TURBELLARIA (flatworm)	NEMATODA (unsegmented worm)	OLIGOCHAETA (segmented worm)	HIRUDINEA (leech)	ISOPODA (sow bug)
	𝍸 𝍸 𝍸	𝍸 𝍸 𝍸 𝍸 𝍸 𝍸 𝍸 𝍸	II		

AMPHIPODA (scud)	DECAPODA (crayfish)	HYDRACARINA (mite)	EPHEMEROPTERA (mayfly)	ODONATA - ANISOPTERA (dragonfly)	ODONATA - ZYGOPTERA (damselfly)
			𝍸		

PLECOPTERA (stonefly)	HEMIPTERA (back swimmer)	MEGALOPTERA (fishfly)	TRICHOPTERA (caddisfly)	LEPIDOPTERA (aquatic caterpillar)	COLEOPTERA (riffle beetle)
𝍸			III		

DIPTERA

Chironomidae (midge larva)	Tabanidae (horsefly)	Culicidae (mosquito)	Ceratopogonidae (no-see-um)	Tipulidae (cranefly)	Simulidae (blackfly)	GASTROPODA (snail)	PELECYPODA (clam)
𝍸 𝍸 𝍸 1		1	II	𝍸 𝍸 𝍸 I			1

APPENDIX 4

EXAMPLE 2: COMPLETED LAKE SURVEY SHEET

Lake / Stream Name: WOLF Stream Code: WFL Site Code: 1 Date: 96·09·20 Crew: J SMITH B LAWRENCE

Technique used: KICK + SWEEP Replicate number: ____ Number of Bottles: 1 Water Depth: ____ Slope (in): ____

Substrates present: Clay: ____ Silt: ____ Sand: 40 Gravel: 30 Cobble: 20 Boulder: 10 Bedrock: ____

SUBMERGED MACROPHYTES 40%

COELENTERATA (hydra)

TURBELLARIA (flatworm)

NEMATODA (unsegmented worm)

OLIGOCHAETA (segmented worm)

HIRUDINEA (leech)

1

ISOPODA (sow bug)

AMPHIPODA (scud)

DECAPODA (crayfish)

HYDRACARINA (mite)

EPHEMEROPTERA (mayfly)

ODONATA - ANISOPTERA (dragonfly)

ODONATA - ZYGOPTERA (damselfly)

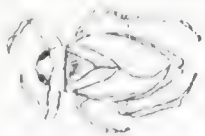
IIII IIII IIII IIII IIII III

IIII IIII I

IIII

II

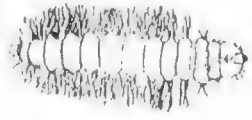
PLECOPTERA (stonefly)



HEMIPTERA (back swimmer)

MEGALOPTERA (fishfly)

TRICHOPTERA (caddisfly)

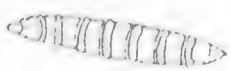


LEPIDOPTERA (aquatic caterpillar)

COLEOPTERA (riffle beetle)

IIII IIII IIII IIII I

Chironomidae (midge larva)



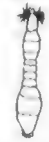
Tabanidae (horsefly)

Culicidae (mosquito)

DIPTERA

Ceratopogonidae (no-see-um)

Tipulidae (cranefly)



Simulidae (blackfly)

GASTROPODA (snail)

PELECYPODA (clam)

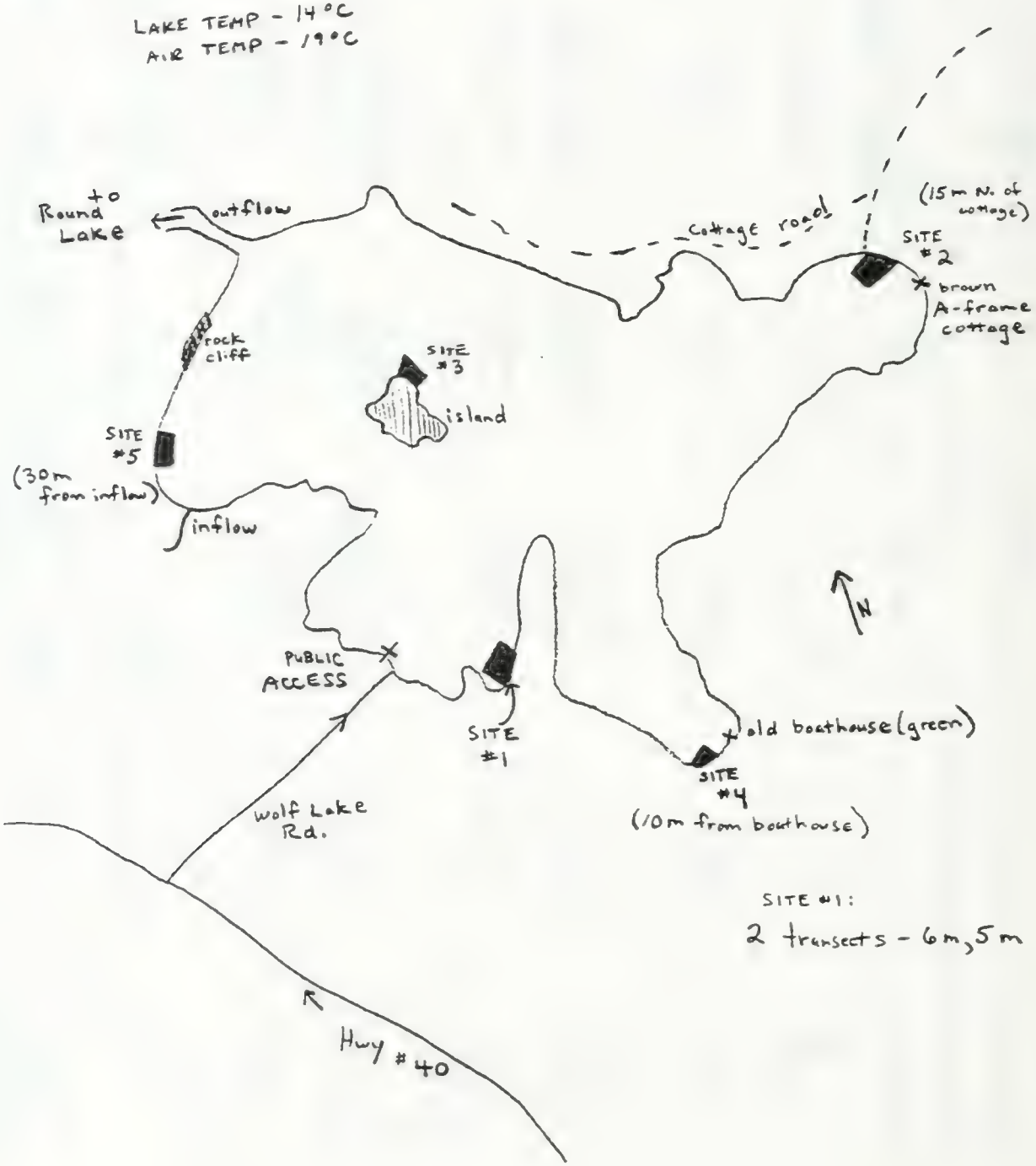
IIII IIII IIII IIII II

IIII

1

TWP : BURLEIGH
NAME: WOLF LAKE
CODE: WFL
DATE : 960920

LAKE TEMP - 14°C
AIR TEMP - 19°C



SITE #1:
2 transects - 6m, 5m

APPENDIX 5

RAPID BIOLOGICAL ASSESSMENTS:

COMPARING PONAR GRABS WITH KICK-AND-SWEEP SAMPLES

elsewhere. A fourth sample was sent to the Dorset Environmental Science Centre where the sediments were subsampled and 100 animals were enumerated using the rapid bioassessment protocol.

Wherever feasible a ten-minute kick-and-sweep sample was also collected from the shallows at the shore in the vicinity of the Ponar-grab samples (i.e., in less than 0.5 m of water). The kick-and-sweep samples were also processed using the protocols in this document. These samples generally required 45 minutes for two people to sort, remove and enumerate a subsample of 100 animals.

For comparative purposes, the bench sheets associated with each site were summarized into the 5 most-common groups: amphipods (or scuds, i.e., small shrimp-like crustaceans); chironomids (midges); ephemeropterans (mayflies); oligochaetes (aquatic worms); and pelecypods (clams, including zebra mussels); plus an "others" category. The abundances in these 6 categories were re-expressed as percentages and plotted as pie diagrams directly on a map of Lake Couchiching.

Results - There was considerable variation in the proportions of the different taxonomic groups collected from the 21 sites with the Ponar grab (Fig. 1). Different groups predominated at different sites suggesting that the 100-animal counts and coarse taxonomic resolution were sufficient to reveal marked differences between the sites. For example, mayflies predominated at some sites, whereas other sites were characterized by high proportions of pollution-tolerant oligochaetes and midges. Correlations between the macroinvertebrate community data and water- and sediment-quality data will be reported elsewhere.

The rapid bioassessment based on the kick-and-sweep protocol also revealed marked differences among the 17 sites that were sampled (Fig. 2). Again, different taxonomic groups predominated at different sites. Moreover, neighbouring sites often supported quite different macroinvertebrate communities suggesting considerable variation around the lake. However, without adequate within-site replication, this variation may simply reflect random noise instead of true biological signals (but see Reid et al. 1995). As a result, further ground-truthing will be necessary to conclusively determine the ecological value of using rapid bioassessment techniques instead of traditional exhaustive censuses.

A simple comparison of the rapid bioassessment results based on these two sampling strategies revealed considerable differences (compare Figs. 1 and 2). For example, ephemeropterans predominated in the kick-and-sweep sample from site 20, but amphipods were most common in the Ponar grab offshore in 2 m of water. Similarly, amphipods were most abundant in the kick-and-sweep sample at site 17, but chironomids were most numerous in the Ponar grab sample.

These differences emphasize that the two sampling methods produce different results. In hindsight, this finding should be expected. The habitats at a 2-m depth are probably quite different from the habitats at the shore. Nearshore areas are affected by wave action, runoff and stream discharges to a greater degree than sites at a depth of 2 m. In addition, the plant communities associated with the two depths differed markedly.

Summary - In a simple comparative study of the ability of a rapid bioassessment protocol to distinguish sites within a large lake in south-central Ontario, we found remarkable differences among neighbouring sites. Simple 100-animal counts and coarse taxonomic resolution were sufficient to distinguish sites and samples collected at different depths using different sampling methods. These results suggest that rapid bioassessment protocols may offer a cost-effective alternative to time-consuming full macroinvertebrate enumerations. Moreover, traditional deep-water sampling strategies can be coupled with 100-animal counts and coarse taxonomy to produce tangible rapid bioassessment results. These findings also underscore the point that grab samples should be compared with grab samples (and kick-and-sweep samples with kick-and-sweep samples) when trying to evaluate the health of a given site relative to reference-area conditions.

RAPID BIOLOGICAL ASSESSMENTS:
COMPARING PONAR GRABS WITH KICK-AND-SWEEP SAMPLES

Rapid bioassessment protocols are often designed to be used by inexperienced volunteers as a way to screen a large number of sites and identify atypical ones as candidates for follow-up studies. The protocols described in the main body of this document are consistent with that objective. Thus, the equipment costs are modest and the targeted sites are shallow wadeable areas of streams and lakes. Because of this narrow focus, deeper bodies of water cannot be evaluated using simple kick-and-sweep methods. For example, large rivers and the deeper areas of lakes fall beyond the scope of these protocols.

In order to sample benthic macroinvertebrates in deeper waters, a number of options are available. An inexpensive method recommended by the New York Department of Environmental Conservation (Bode et al. 1990) involves the use of artificial substrates. An artificial substrate sampler is constructed from a series of tempered hardboard squares that are separated by spacers on a turnbuckle (also see Klemm et al. 1990). Several of these devices are suspended from buoys at some standardized depth in the area of interest. After 5 weeks the substrates are retrieved and the invertebrates that have colonized the squares are enumerated and compared to samples from artificial substrates deployed at reference areas.

Artificial substrates have the advantage of being inexpensive, easily constructed and simple to use. In addition, the hardboard surfaces provide a standardized "habitat" for the invertebrates to colonize. However, there are several drawbacks. One concern lies in the type of animals that will colonize artificial substrates. These animals do not represent the full benthic community. Moreover, factors such as the length of time for colonization, the season when they are used, plus the depth and current will all affect the types of animals that are collected. As a result, bench sheets based on artificial substrates should only be evaluated through comparisons with data from artificial substrates deployed in a similar manner in areas with acceptable water quality.

Alternatively, traditional bottom-sampling equipment such as grabs and corers can be used to sample deep-water sites. Klemm et al. (1990) provide pictures and descriptions of a number of these sorts of devices. In rigorous scientific studies of macroinvertebrate communities, these types of samplers are preferred because they provide quantitative estimates of animal abundances on a per-unit-area basis. However, many of these devices are expensive and some require specialized boats with winches if they are to be used safely. As a result, grabs and corers are often impractical for inexperienced volunteers to use. Despite these requirements, many monitoring agencies prefer grab samplers instead of kick-and-sweep methods. This appendix compares the results from Ponar-grab samples with those from nearshore kick-and-sweep samples.

Methods - Recently we assisted biologists from the Environmental Monitoring and Reporting Branch and the South-Western Region of the Ontario Ministry of the Environment in an effort to field-test these rapid bioassessment protocols. As part of a survey of the current status of Lake Couchiching near Orillia, Ontario, grab samples were collected from a number of sites around the lake. Most of these samples were collected in about 2 m of water using a Ponar grab. Three additional sites were sampled in the middle of the lake at depths ranging from 7 to 9 m. Triplicate samples were saved for full sorting and identification to the genus and species levels. These results will be reported

Figure 1: Rapid Bioassessment of Lake Couchiching Ponar-Grab Samples

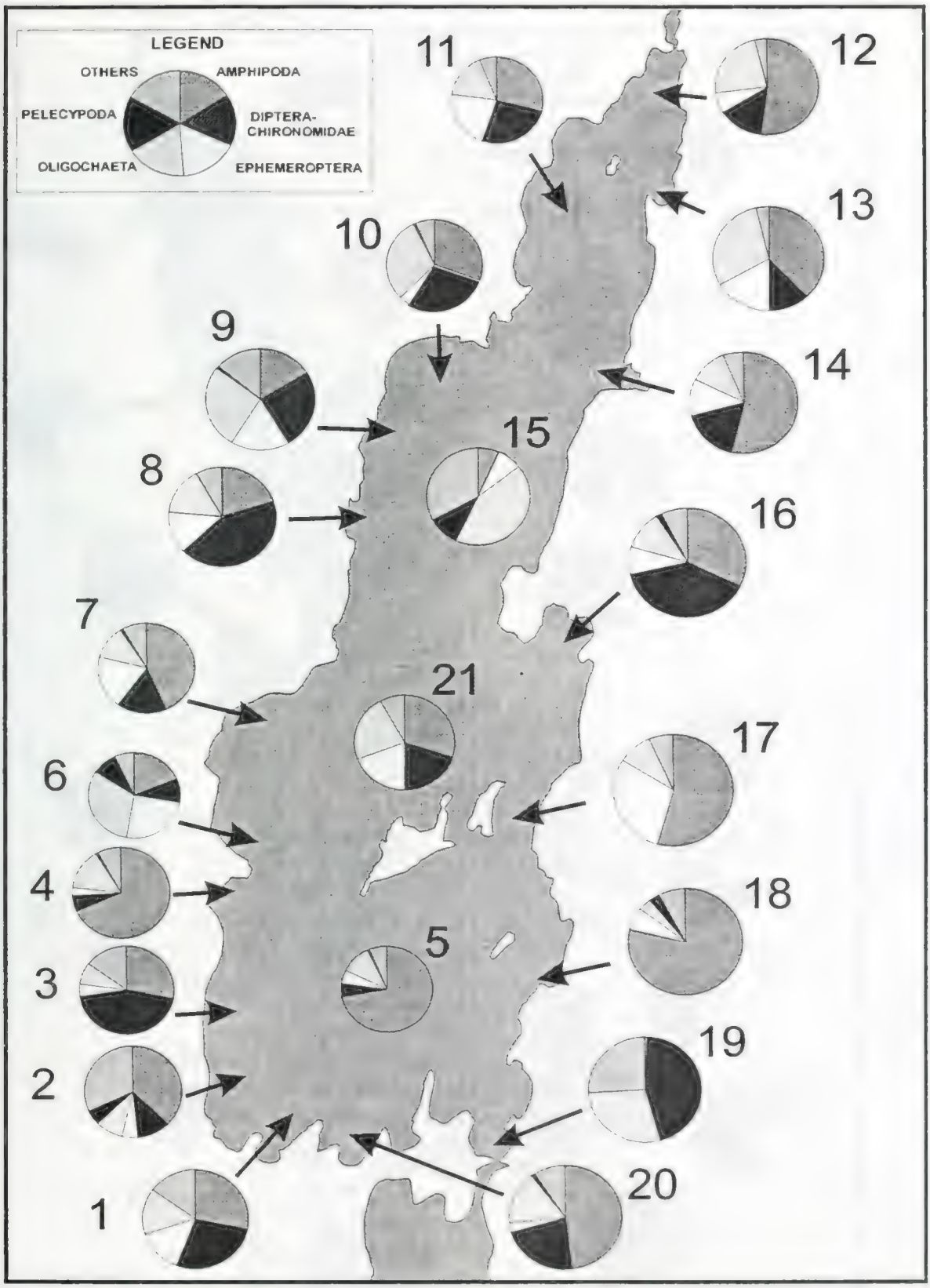
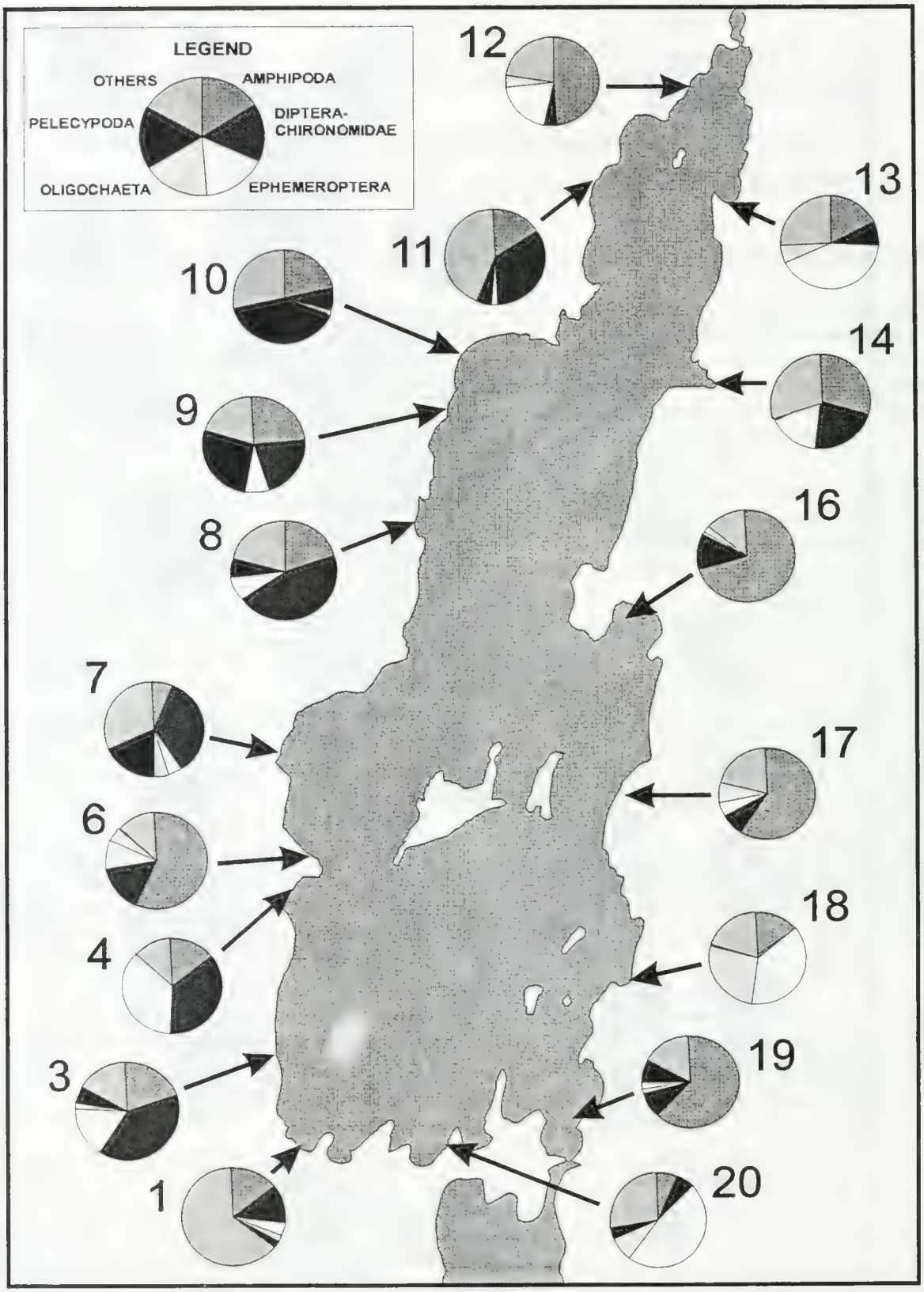


Figure 2: Rapid Bioassessment of Lake Couchiching Kick-and-Sweep Samples

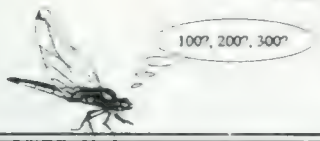


APPENDIX 6

RAPID BIOLOGICAL ASSESSMENTS:

HOW MANY ARE ENOUGH?

RAPID BIOLOGICAL ASSESSMENTS
HOW MANY ARE ENOUGH?



Many protocols have been developed for rapid biological assessments to evaluate the health of lakes and streams. Our protocol involves a simple kick-and-sweep approach where the investigator wades out to a depth of about one metre, kicks the sediments to disturb the macroinvertebrates and then captures these animals by sweeping a net through the water column. Five sites are each sampled for 10 minutes in a given lake. The resultant debris and associated macroinvertebrates from each site are randomly subsampled until a total of 100 animals have been removed, identified and enumerated. Most rapid bioassessment protocols require the enumeration of 100 macroinvertebrates. Because this number is arbitrary, there is some debate regarding the optimum number of animals that should be counted. Many ecologists believe that smaller impacts can be detected if more animals are counted. This appendix examines the question: "How many are enough?"

Methods - Recently we sampled a number of lakes that span a gradient of recreational cottage densities in south-central Ontario. Five of these lakes were selected for the current study. The debris collected from each of the 5 sites in each lake was subsampled using the standard protocol described above. The remaining debris was then randomly subsampled a second and third time to obtain 2 additional sets of 100 animals. The numbers on the first sheet for a given site were used for the traditional 100-count assessment. Data on the first and second bench sheets were combined to provide a 200-count total, and the numbers on all 3 sheets were summed to obtain a 300-animal count. This approach provides results that are consistent with counting a total of 100, 200 or 300 animals.

The resultant counts were converted to a series of rapid bioassessment indices (see Table 1). In addition to these simple metrics, we also used the data in a correspondence analysis ordination (CA) to provide 3 multivariate metrics based on the first 3 ordination axes (i.e., Abundance CA Axis 1, 2 and 3). This analysis was repeated with simple presence-absence data to provide 3 additional multivariate metrics (i.e., Presence - Absence CA Axis 1, 2 and 3). These 6 indices are objective summaries of the multi-taxa nature of the benthic communities.

We used a simple one-way analysis of variance (ANOVA) to assess the ability of the different indices to separate the 5 lakes. Results of each ANOVA were tabulated as F values with the associated probability (i.e., the P value) plus the intraclass correlation (r_I) which ranges from -1 to +1. The intraclass correlation can be interpreted as the proportion of the total variation associated with differences among the 5 lake means (i.e., the among-lake variance component). The intraclass correlation is calculated using the means squares from the one-way ANOVA as:

$$(MS_{lakes} - MS_{error}) / (MS_{lakes} + [n_{repl} - 1] \times MS_{error}).$$

A negative intraclass correlation indicates that within-lake variation was so large that the among-lake variance component could not be distinguished from zero.

Table 1. Median, first and third quartiles for the simple, rapid bioassessment indices for each of the 3 counting protocols based on data for 5 sites in each of 5 lakes.

Rapid Bioassessment Index	100-Count			200-Count			300-Count		
	Median	Percentile		Median	Percentile		Median	Percentile	
		25th	75th		25th	75th		25th	75th
Number of Groups	8	7	9	10	9	11	11	10	12
% Worms	0.00	0.00	0.01	0.00	0.00	0.01	0.00	0.00	0.01
% Amphipods	0.23	0.05	0.50	0.23	0.04	0.47	0.21	0.04	0.45
% EPTs	0.13	0.09	0.25	0.15	0.11	0.27	0.14	0.11	0.28
% Non-Dipteran Insects	0.27	0.16	0.41	0.26	0.18	0.38	0.27	0.18	0.41
% Insects	0.76	0.50	0.90	0.77	0.50	0.91	0.78	0.52	0.92
% Dipterans	0.43	0.28	0.55	0.45	0.26	0.56	0.44	0.27	0.54
% Snails	0.00	0.00	0.01	0.00	0.00	0.01	0.01	0.00	0.01
% Clams	0.00	0.00	0.02	0.00	0.00	0.01	0.01	0.00	0.01
EPTs/Midges	0.25	0.17	0.46	0.28	0.17	0.52	0.29	0.18	0.56
% Dominants	0.50	0.48	0.59	0.51	0.47	0.58	0.50	0.46	0.58

The one-way ANOVA results were also used to estimate the power of these comparisons. In this context, power indicates the probability of correctly concluding that there was a significant difference among the 5 lakes at the traditional alpha (Type-I error) level of $P \leq 0.05$. For comparative purposes, power values were standardized and expressed as the minimum detectable change (MDC). As such, MDC represents the difference among the means that could be detected at $P \leq 0.05$ with 80% power divided by the observed range among the 5 lake means. Thus, a MDC of 0.5 indicates that the ANOVA could detect a difference of one-half the range between the largest and smallest means 4 out of 5 times.

These steps were repeated for the 100, 200 and 300 counts. If larger subsamples are better, we would expect to see more significant ANOVA results with the 300-count data. Similarly, we would expect to see larger MDC values for the 100-count data. By comparing results for the different indices we can also determine which indices best separate the 5 lakes.

Results - Medians with first and third quartiles for the simple indices are presented in Table 1. Several indices frequently produced zero values, suggesting that these indices were of limited use in distinguishing the 5 lakes. These metrics included % worms, % snails and % clams. The medians of most of the indices changed very little when more animals were counted. Exceptions included the number of taxonomic groups, the percent insects and the ratio of EPTs/Midges, which all increased with larger counts. For most indices, the inter-quartile range (i.e., the difference between the 25th and 75th percentiles) showed virtually no change with increasing counts. The absence of clear trends suggests that counting more animals has limited value.

The ordinations of the 100, 200 and 300-count data also varied modestly (Fig. 1). The 5 sites from each lake tended to cluster together although each lake generally overlapped with at least one other lake. Differences in the total counts did not translate into greater separation among the lakes (i.e., compare Figs. 1A, C and E). The important taxonomic groups in each ordination also changed very little with increasing counts (as indicated by the length and direction of the arrows; compare Figures 1B, D and F). The first CA axis separated Dickie and Basshaunt lakes at the positive end of the axis from Moot and Chub lakes at the

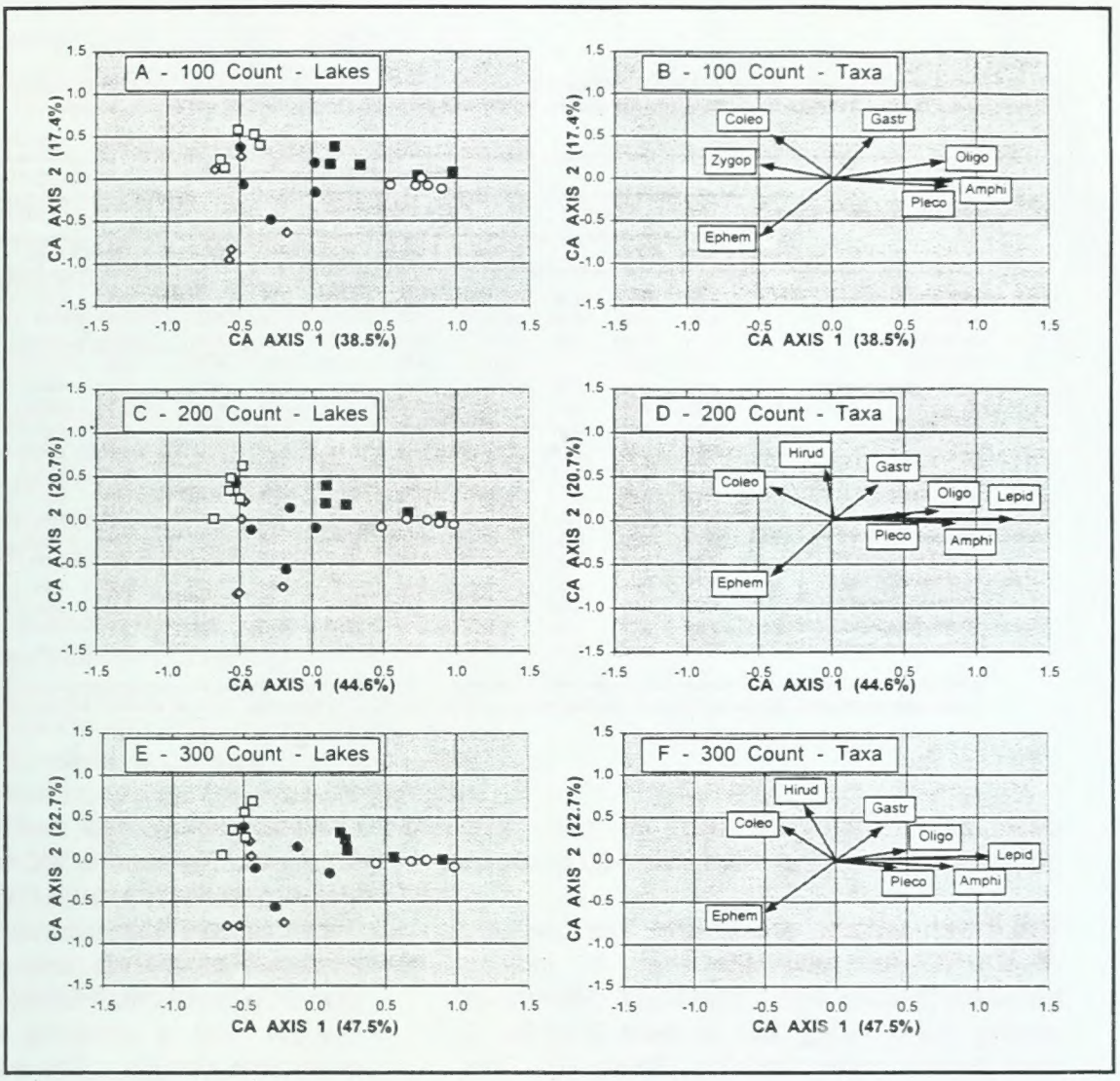


Figure 1. Results of the correspondence analysis ordinations of the 100, 200 and 300 count data for the 5 sites in each of the 5 lakes.

negative end. Dickie and Basshaunt lakes had the greatest proportions of the oligochaetes (worms), lepidopterans (aquatic butterfly larvae), amphipods and plecopterans (stoneflies). Moot and Chub lakes were separated along the second CA axis. Chub Lake had proportionately more ephemeropterans (mayflies), whereas Moot Lake was characterized by a larger proportion of coleopterans (aquatic beetles), and zygopterans (damselflies). The lakes exhibited considerably more overlap in the ordination of the presence-absence data (not shown), although the relative positions of the sites and the taxa changed very little when more animals were counted.

Is more better? Individual metrics revealed different trends as more animals were counted (Table 2). For example, 5 indices (i.e., % worms, % clams, % dominants, and the first and second axes of the presence - absence CA ordination) never detected a significant difference among the 5 lakes. By contrast, 8 metrics always indicated significant differences for the 100-, 200- and 300-count

Table 2. Observed one-way ANOVA F values and associated probabilities (P values) of a significant difference among the 5 lakes. The intraclass correlation (r_i) and the minimum detectable change (MDC) are also provided.

Rapid Bioassessment Index	One-Way ANOVA RESULTS											
	100-Count				200-Count				300-Count			
	F Value	P Value	r_i	MDC*	F Value	P Value	r_i	MDC	F Value	P Value	r_i	MDC
Number of Groups	4.07	0.01	0.38	1.04	1.59	0.22	0.11	1.82	1.86	0.16	0.15	1.45
% Worms	0.44	0.78	-0.13	3.59	0.77	0.55	-0.05	2.23	0.42	0.79	-0.13	3.24
% Amphipods	32.21	<0.001	0.8[illegible]	0.40	30.31	<0.001	0.85	0.41	30.02	<0.001	0.85	0.41
% EPTs	7.47	<0.001	0.[illegible]	0.81	8.75	<0.001	0.61	0.75	9.41	<0.001	0.63	0.72
% Non-DipteranInsects	8.55	<0.001	0.60	0.77	9.48	<0.001	0.63	0.73	10.23	<0.001	0.65	0.71
% Insects	22.38	<0.001	0.81	0.51	24.14	<0.001	0.82	0.48	24.34	<0.001	0.82	0.48
% Dipterans	4.70	0.01	0.43	0.94	4.39	0.01	0.40	0.97	4.08	0.01	0.38	1.00
% Snails	0.87	0.50	-0.03	2.13	3.21	0.03	0.31	1.24	2.47	0.08	0.23	1.40
% Clams	2.16	0.11	0.19	1.41	1.66	0.20	0.12	1.67	2.45	0.08	0.23	1.36
EPTs/Midges	5.40	0.01	0.4[illegible]	0.92	5.20	0.01	0.46	0.95	5.46	0.01	0.47	0.95
% Dominants	1.17	0.35	0.0[illegible]	1.97	0.76	0.56	-0.05	2.44	0.96	0.45	-0.01	2.21
ABUNDANCE CA AXIS 1	30.46	<0.001	0.85	0.46	33.54	<0.001	0.87	0.41	34.96	<0.001	0.87	0.41
ABUNDANCE CA AXIS 2	4.42	0.01	0.41	0.96	4.59	0.01	0.42	0.95	4.60	0.01	0.42	0.93
ABUNDANCE CA AXIS 3	2.42	0.08	0.22	1.39	3.82	0.02	0.36	1.22	3.17	0.04	0.30	1.34
PRESENCE-ABSENCE CA AXIS 1	1.30	0.31	0.06	1.94	2.17	0.11	0.19	1.53	1.88	0.15	0.15	1.48
PRESENCE-ABSENCE CA AXIS 2	2.63	0.07	0.25	1.36	0.77	0.56	-0.05	2.55	2.14	0.11	0.19	1.73
PRESENCE-ABSENCE CA AXIS 3	2.11	0.12	0.18	1.40	4.42	0.01	0.41	1.04	1.04	0.41	0.01	2.17

* - MDC - Minimum Detectable Change expressed as the detectable difference divided by the range of the between-lake means with alpha=0.05 and power=0.80

data. Generally, there was no co[illegible]tent advantage in counting more than 100 a[illegible]nals.

Which indices are best? The one-way ANOVAs revealed that 8 indices generally failed to detect significant differences among the 5 lakes (i.e., at least 2 of the 3 comparisons with P>0.05, Table 2). The intraclass correlations for these same metrics were generally less than 0.25 which indicates that less than 25% of the variation was attributed to differences among the lakes. In several instances, the intraclass correlations were negative revealing considerable variation among the sites within the lakes. In addition, the minimum detectable change for these 8 indices exceeded the range between the largest and smallest mean (i.e., MDCs larger than 1.0). Consequently, 8 of the metrics had limited power to detect differences among the lakes.

The results also show that some metrics were better than others (Table 2). The largest F and r_i values, and smallest minimum detectable changes characterize the best indices. The % amphipods, the first axis from the CA ordination of the abundance data, and % insects were the 3 best metrics for the 100-count data. There was a marked drop in the F values for the other 6 metrics which corresponds with a drop in the among-lake variance component from greater than 80% to less than 60% (i.e., from $r_i \geq 0.80$ to $r_i \leq 0.60$) and a doubling of the minimum detectable change. The success of these 3 metrics is not surprising, since a decline in the numbers of amphipods and an increase in midges have been widely recognised as indicators of water-quality impairment due to acid rain. In addition, multivariate summary indices (such as Abundance CA 1) have also proven useful in many studies.

A simple correlation analysis revealed that several of these in[illegible]ces were redundant. For example, the 3 [illegible]est metrics were inter-correlated (i.e., $|r| > 0.985$). A decline in the percent amphipods [illegible]erally coincided with an increase in chirono[illegible]s. Similarly, % EPTs was highly correlated with % non-dipteran insects (r=0.918) and the EPTs/midges ratio (r=0.923). These latter correlations are not surprising since the EPTs comprise a large

proportion of the insects that are not flies (i.e., not dipterans), and the EPT index is the numerator of the ratio with the number of midges. Given the high redundancy among these indices, simple metrics such as the percent amphipods or the percent insects, or the multivariate index (i.e., Abundance CA Axis 1) would be sufficient to characterize these lakes. Several of the other indices such as the EPT index also have a long history of use in assessments of water quality.

Summary -Despite the common perception that more is better, the doubled or tripled effort required to sort, identify and enumerate more animals resulted in very little improvement in our ability to distinguish lakes (i.e., <5% maximum). Based on this comparative analysis, subsamples using counts of 100 animals are sufficient to distinguish the littoral benthic communities of small inland lakes in south-central Ontario. High correlations among the best 6 indices suggested that one-or-two metrics would be sufficient to characterize these lakes. Three indices including the percent amphipods (an established indicator of acid-rain impacts), the percent insects (largely midges which are also an acid-rain indicator), and the first axis from a correspondence analysis ordination were recommended.